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RADIATION PNEUMONITIS AND FIBROSIS: A COMPLICATION OF RADIOIODINE TREATMENT OF PULMONARY METASTASES FROM CANCER OF THE THYROID*

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INTRODUCTION

THIS paper has been prompted by the observation that large doses of radioiodine given to patients with pulmonary metastases from thyroid cancer may produce severe pulmonary damage. Although this is a complication of radioiodine therapy heretofore unreported, it has been observed following roentgen irradiation directed to the chest. From the latter studies, the pathologic anatomy of radiation-induced pulmonary fibrosis and a rough notion of the dosage of x-ray irradiation required to produce it, has been obtained (1, 2). The present report describes in some detail the histories of 2 patients in whom fatal pulmonary fibrosis and pneumonitis developed. It also describes briefly our experience in other patients with pulmonary metastases from carcinoma of the thyroid who received one or more large doses of I^{131}. Two of these patients have symptoms of pulmonary insufficiency, probably secondary to pulmonary fibrosis.

CLINICAL MATERIAL AND METHODS

Radioiodine as I^{131} was given orally in doses ranging from 107 mc. to 355 mc. All doses were in millicuries conforming to the National Bureau of Standards. In previous

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publications from this laboratory, the New York Hospital physicists' "millicurie" was used, which is equivalent to 1.2 millicuries in terms of the National Bureau of Standards. Urine and blood were analyzed for radioactivity as described previously (3).

Readings over the neck and over the metastases were made at frequent intervals. In general, it is difficult to determine quantitatively *in vivo* the uptake of I^{131} in the lungs. This is particularly true if other metastases pick up iodine or if there is residual thyroid tissue in the neck. When there was uptake only in the lungs or in the lungs plus the thyroid, the following appeared to be the most reasonable way to approximate the millicuries deposited in the lungs twenty-four hours after the dose of I^{131}:

$$L_{mc} = .9D - (U_S + N.)$$

where L_{mc} = mc. in lungs at twenty-four hours; D = Number of mc. administered; U_S = asymptotic urinary excretion; and N. = thyroidal uptake at twenty-four hours. The asymptote of the urinary excretion is the value observed in eight to forty-eight hours, and represents completion of iodide excretion. Since most of these subjects had functionally active tumors, this asymptote was reached at the time usually noted in euthyroid or hyperthyroid individuals. The factor of 0.9D was used because, in general, at forty-eight hours about 10 per cent of the dose is not accounted for by these measurements and is presumably in the blood and gastro-intestinal tract. Obviously, variations in thyroid and renal iodide clearance markedly alter this fraction. Furthermore, this calculation does not take into account the biologic life of I^{131} in the tumor, which markedly affects the total radiation delivered.

For 10 doses in 9 patients, it was possible to determine the integral amount of I^{131} in the chest. These patients had uptake either in the chest alone, or in the chest plus a discrete area of the neck. "Curie days" were calculated by integrating with a planimeter a graphic plot of millicuries in the chest as determined daily for twenty days. The amount in the chest was calculated by subtracting the amount excreted into the urine

TABLE 1

Patient	Age (yrs.)*	Sex	Metabolic status†	Pulmonary metastases	Other metastases
W.Mc.	15	M	Eu	Diffuse marked	None
G.F.	27	F	Eu	Diffuse moderate	None
O.D.	32	F	Hypo	Diffuse moderate	None
L.F.	13	F	Hypo	Diffuse moderate	Neck
J.P.	14	F	Hypo	Diffuse moderate	Mediastinum
T.G.	20	M	Hypo	Diffuse moderate	None
A.G.	48	F	Hyper	Discrete nodules	None
P.L.	24	M	Hypo	Diffuse moderate	None
G.G.	32	F	Eu	Discrete nodules	Pelvis, jaw
M.K.	50	F	Hyper	Diffuse marked	None
L.L.	60	F	Hypo	Diffuse moderate	Clavicle, neck
I.M.	54	F	Eu	Diffuse moderate	Pelvis, spine, femur
J.D.	46	F	Hypo	Diffuse moderate	Pelvis, spine
S.F.	52	M	Hypo	Diffuse and discrete	Neck
D.K.	15	F	Hypo	Diffuse moderate	None

* At time of first dose.

† Eu = euthyroid; Hypo = hypothyroid; Hyper = hyperthyroid.

up to that time plus the amount in the blood at that time, from the administered dose. The I^{131} in the blood was assumed to have a volume of distribution of 40 per cent of the body weight for the first exponential rate of decay, and a volume of 10 per cent of the body weight for the first build-up and decay.

Fifteen patients with pulmonary metastases were given I^{131} in therapeutic doses. The number of doses per patient ranged from 1 to 5. Some patients had been previously

TABLE 2

Patient	Total dose of I^{131} (mc.)	Dose to chest* (mc.)	Time (yrs.)†	Effect on metastases‡	Pulmonary damage§
W.Mc. 1)	134	127	8.0	+1	0
2)	128	102	5.0	+2	2
G.F.	300	193	0.3 (d)	+1	4
O.D.	212	162	0.3 (d)	0	4
I.F.	300	210	2.9	+1	3
J.P. 1)	180	69	6.0	0	
2)	350	26	2.3	−1	0
T.G. 1)	152	119	0.6	+1	1
2)	241	125	5.0	+2	1
A.G.	285	174	6.0	+2→−1	0
P.L. 1)	295	97	4.5	0	0
2)	362	54	3.8	+1	2
G.G.	180	44	5.3	+2	0
M.K.	238	43	0.3 (d)	−2	0
L.L.	238	79	1.8	?	?
I.M. 1)	239	<121?	2.7 (d)	+1	0
2)	209	×164?	2.2 (d)	+2	0
J.D. 1)	302	<133	3.1 (d)	+1	0
2)	208	< 55	2.7 (d)	+2	0
S.F.	355	84	1.7 (d)	+1→−2	0
D.K. 1)	129	110	5.7	0	0
2)	252	48	3.0	+1	3

* See text for calculations.

† Interval from dose until Dec. 1956 or until death (d).

‡ Effect: −2 = progression to death; −1 = progression; 0 = no change; +1 = improvement; +2 = disappearance.

§ Damage: 0 = none; 1 = minimal x-ray reaction; 2 = moderate x-ray reaction; 3 = moderate x-ray reaction + symptoms; 4 = death from pulmonary insufficiency.

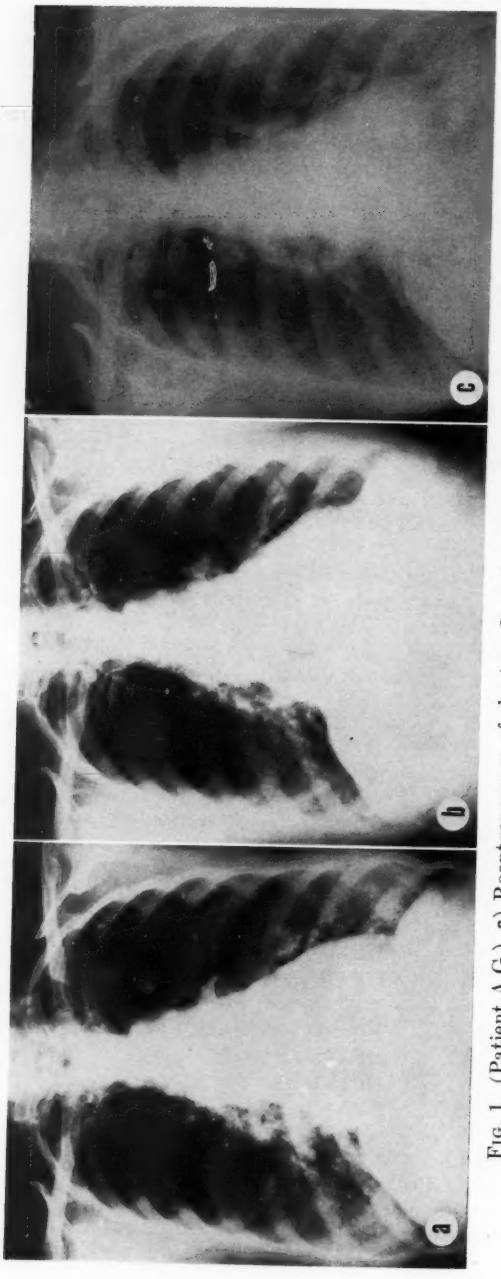

FIG. 1. (Patient A.G.). **a)** Roentgenogram of chest on Dec. 6, 1950, two weeks before a therapeutic dose of I^{131}; **b)** April 1954; **c)** Feb. 1957.

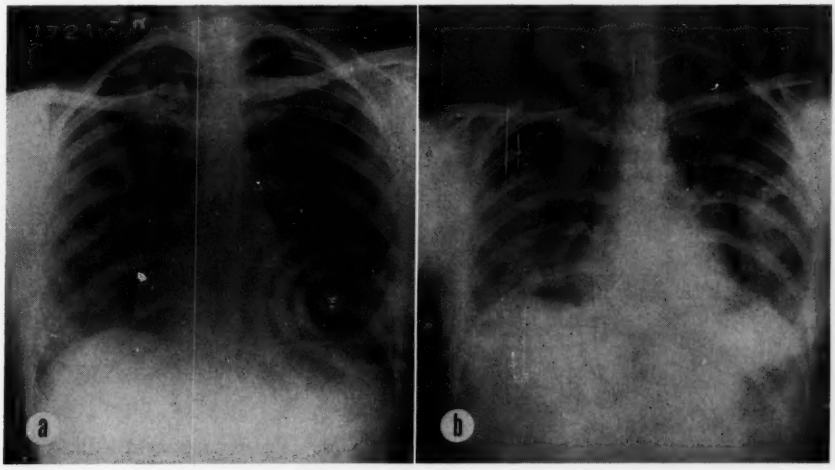

FIG. 2. (Patient O.D.). a) Roentgenogram of chest in June 1953, just prior to treatment with I^{131}; b) two weeks prior to death on Sept. 27, 1953.

thyroidectomized and some had residual thyroid function. Prior to I^{131} therapy, some of the patients had been treated with a thiouracil derivative or mercaptoimidazole.

RESULTS

In Table 1 are listed certain clinical data regarding the 15 patients treated; the age and clinical status are those at the time of the first dose of I^{131}. Table 2 shows the doses of I^{131} (in millicuries) and the frequency of radiation fibrosis. It can be seen that neither hypothyroidism nor hyperthyroidism appeared to affect the development of pulmonary fibrosis. The character of the pulmonary metastases, as might be expected, did seem to be important. Patient A.G., for example, had isolated discrete pulmonary lesions; in spite of a chest dose of 174 mc., pulmonary fibrosis did not develop. Roentgenograms of her chest before, and at two intervals after I^{131} therapy are shown in Figure 1: a) in December 1950 just before she received the therapeutic dose; b) in April 1954, showing essentially complete regression of the metastases (the x-ray findings being read as negative); and c) in February 1957, showing recurrence of metastases seven years after the initial therapy. Pessimism about the long-term results of treatment of cancer of the thyroid with I^{131} was confirmed in this case. On the other hand, Patients O.D. and G.F. (see Appendix) both of whom died from radiation pneumonitis after chest doses of 162 mc. and 193 mc. respectively, had multiple fine, diffuse pulmonary lesions (Figs. 2 and 3). Figure 2b shows the appearance of the chest on an x-ray film during the radiation pneumonitis. Large patchy infiltrates were present

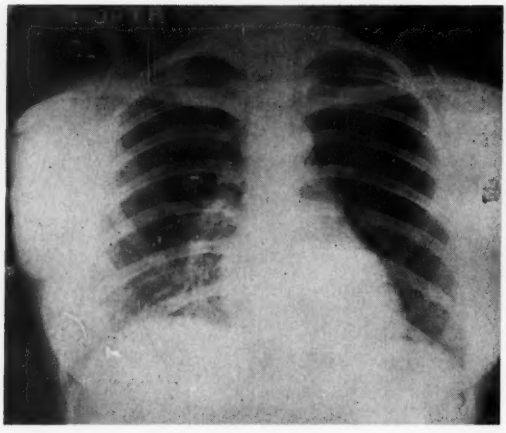

FIG. 3. (Patient G.F.). Roentgenogram of chest just prior to treatment with I^{131}.

in both lung fields. Detailed case histories of these 2 cases may be found in the appendix. It is not clear whether the large diffuse "snow-storm" metastases differed from the finer metastases. In Patient W.M. who had large (1 cm.) metastases, x-ray evidence of pulmonary fibrosis developed after doses which deposited 127 mc. and 102 mc., in the lungs, but the fibrosis was not sufficient to cause clearly discernible physiologic changes in pulmonary capacity. The status of this patient, however, was complicated because of coexistent rheumatic heart disease with mitral stenosis and insufficiency. Patient L.F. appeared to be an exception to any pattern, since she had the largest chest dose of I^{131} in the series and her pulmonary metastases were diffusely located. No more than minimal x-ray evidence of pulmonary fibrosis developed. Figure 4 shows roentgenograms of the chest in this patient, before and after treatment. In the latter, evidence of extensive metastases had virtually disappeared and had been replaced by a fine network that probably represents fibrotic changes. Pulmonary function studies showed that after an initial low value for vital capacity, there was a gradual increase to virtually normal values over the course of eighteen months.

The results of radiodine treatment of pulmonary metastases from a therapeutic standpoint can be seen in Table 2. In 11 of the 15 patients a decrease in the size, or disappearance of the lesions was evident upon x-ray examination. This improvement was permanent within the time of study in 9, but in 2 there was a recurrence. In Patient S.F. the recurrence was noted about a year after treatment, whereas in Patient A.G. there was no definite evidence of recurrence until five years and nine months after treatment.

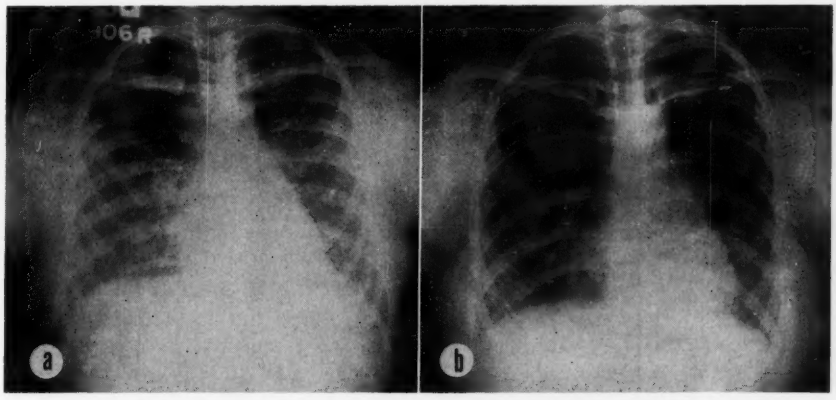

FIG. 4. (Patient L.F.). a) Roentgenogram of chest prior to treatment with I^{131}; b) four years after treatment.

Figure 5 shows the relationship between the dose of I^{131} deposited in the chest at twenty-four hours and the effect on pulmonary function. It appears that a dose of more than 100 mc. is required to produce discernible changes in pulmonary function. The data are not conclusive, but it does not seem possible to add in simple arithmetic fashion the damage from each of several doses of I^{131}. For example, 8 patients each had a total of 150 mc. or more deposited in the lungs; of this group, 3 received the radiation from a single dose of I^{131} and 5 from several doses. Both patients who

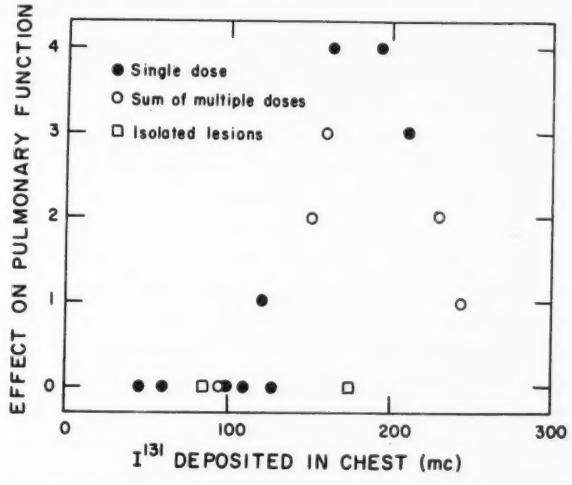

FIG. 5. Relationship between dose of I^{131} deposited in the chest and pulmonary function—at twenty-four hours.

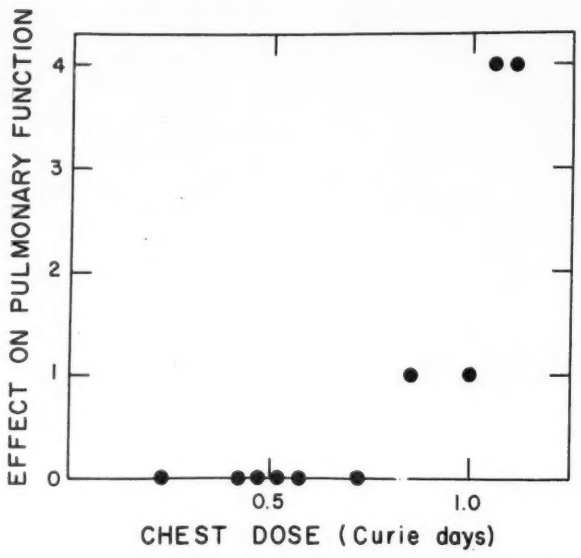

FIG. 6. Relationship between dose of I^{131} deposited in the chest and pulmonary function—calculated for twenty days.

died, and 1 of the 2 with symptoms, received a single dose. Although the multiple-dose patients received as much or more total radiation, only 1 had symptoms of pulmonary damage. It appears, therefore, that 2 doses of 100 mc. of I^{131} deposited in the chest at widely spaced intervals are not as likely to be damaging to normal lung tissue as a single dose of 200 mc. The spacing of the doses is probably of importance. It has been shown that, at least as far as lethality is concerned, there is recovery from irradiation. This recovery probably takes place in exponential fashion, with a half-time of about eight days in the rat and two weeks in the dog (4, 5). Unfortunately, complete recovery is never achieved. In the rat there may be recovery of about 90 per cent (6). The rate of recovery and the absolute magnitude of the reparable damage is not known in the human. It is likely that the rate of recovery is considerably slower than that in rats or dogs. If it proceeded at a rate of from 1 to 1.5 per cent per day, it would require about six months for recovery of approximately 90 per cent of the reparable fraction of the damage. This seems to be a reasonable approximation, and we have therefore attempted to restrict the administration of these large doses to intervals of six months or more. In all multiple-dose cases reported here, an interval of at least six months was allowed between doses.

Figure 6 shows the relationship between the dose of I^{131} deposited in the

chest (calculated for twenty days) and the development of pulmonary damage. Fewer cases were available for these calculations but it seems that there was a somewhat better dose-response relationship when the radiation to the chest was calculated in this manner. No attempt has been made to calculate the rads delivered to normal lung tissue, because of uncertainty regarding the relative importance of beta and gamma radiation and the great difficulty in any estimate of geometrical factors. On the basis of comparison of these data with the results after x-ray irradiation of the normal lung (in which dosage is still a problem but perhaps less complicated) it would appear that a dose of 100 mc. of I^{131} deposited in the chest delivers an average dose of between 1,000 and 2,000 rads.

In Table 3 is summarized the clinical pulmonary status of the entire group of patients prior to treatment and after treatment (up to the present or at the time of death). It can be seen that only 6 of the 15 had symptoms referable to the lungs prior to treatment. Among these 6, following I^{131} therapy, 4 manifested definite symptomatic improvement in pulmonary functional capacity. This clinical improvement was well mirrored by

TABLE 3

Patient	Pulmonary Symptoms	
	Before treatment with I^{131}	After treatment with I^{131}
W.Mc.	+	0
G.F.	0	+++
O.D.	0	+++
L.F.	—	+→0
J.P.	0	0
T.G.	0	0
A.G.	+[1]	0
P.L.	+	+→0
C.G.	0	0
M.K.	++	[2]
L.L.	0	0
I.M.	0	0[3]
J.D.	0	0[3]
S.F.	+	0→++[3]
D.K.	0	+

+ = exertional dyspnea not incapacitating.
++ = dyspnea at rest.
+++ = death from pulmonary insufficiency.
[1] = severe hemoptyses before I^{131} therapy.
[2] = died at home; no autopsy.
[3] = dead from other causes.

changes in the vital capacity, measurements of which were performed in most of the cases. One (M.K.) of the 6 patients died, apparently without having experienced significant improvement; and 1 (S.F.), after improving for a short time, suffered a relapse and finally died. In the entire group, at the time of this writing or at death, only 4 had clinical signs of pulmonary insufficiency.

The hematologic changes after treatment with large doses of I^{131} have been reported before (3). In this series, there were no permanent hematologic changes, although marked leukopenia and thrombocytopenia were seen in many instances. The thrombocytopenia (0 to 20,000 platelets) persisted for as long as three to six months in a few of the cases.

Treatment of pulmonary insufficiency was, in general, disappointing. In 1 of the 2 patients who died, cortisone elicited definite symptomatic improvement, although the vital capacity was not affected. No patients with residual fibrosis have experienced sufficiently severe symptoms up to the time of writing to necessitate any treatment.

From a practical standpoint, we have evolved the following criteria, based on rather tenuous evidence:

1) In a patient with multiple diffuse pulmonary metastases the amount of I^{131}-irradiation delivered to the chest should not exceed 125 millicuries in any single dose.

2) Multiple doses should be separated by an interval of at least six months.

3) The magnitude of any contemplated dose should be further restricted by hematologic considerations, as described in earlier reports (3).

SUMMARY

The course of cancer of the thyroid with pulmonary metastases treated with radioiodine has been reviewed in 15 cases. Two patients died from what was presumed to be radiation pneumonitis. In 4 other patients there was x-ray evidence of pulmonary fibrosis. Nine patients are still alive one and a half to eight years after I^{131} therapy of pulmonary metastases from carcinoma of the thyroid.

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APPENDIX

Patient G.F., ♀

In 1930 at age 4, this patient's left orbit was exenterated for retinoblastoma. The follow-up revealed nothing abnormal over a ten-year period. In May 1941 a mass was noted in the left neck, and six months later the patient underwent a left hemithyroidectomy and left radical neck dissection. The pathologic diagnosis was papillary adenocarcinoma of the thyroid with cervical metastases. In the ensuing four years, three additional operations on the neck, including a right radical dissection, were performed for the removal of recurrent masses. In 1945 a course of 3,600 r to each of two cervical ports was administered. A roentgenogram of the chest at that time showed no metastases. The patient was well for eight years, and despite the apparently complete removal of the thyroid gland she remained euthyroid without replacement therapy.

In January 1953 a supracervical hysterectomy was performed because of leiomyomata. At that time an x-ray film of the chest (Fig. 3) showed numerous nodular densities involving the lower two-thirds of both lung fields. There were no respiratory symptoms. In a 48-hour radioiodine tracer study, the uptake over the neck was 10.5 per cent of the administered dose; the uptake was unequivocal over each lung area. In March 1953, the patient received a dose of 86 mc. of I^{131} for the purpose of completing the thyroidectomy prior to eventual treatment of the metastatic lesions. Two months later she was still clinically euthyroid. Methimazole (Tapazole) was started in a dosage of 45 mg. per day. She then noted slight dryness of the skin, a mild increase of intolerance to cold, and a 15-pound gain in weight, but she felt generally well. She had no respiratory complaints except shortness of breath on climbing stairs.

Four months later she was admitted to the James Ewing Hospital for treatment. She appeared to be in excellent health. The skin was slightly dry. There was no evidence of disease in the neck. The lungs were clear to percussion and auscultation, except for a slight prolongation of expiration. Vital capacity was 105 per cent of normal. BMR's were −27 and −30 per cent. The serum cholesterol level was 280 mg. per 100 ml. A radioiodine tracer study revealed a marked increase in the uptake of the isotope in the chest. On October 22, 1953, the patient received 300 mc. of radioiodine, of which 6 per cent was concentrated in the neck at twenty-four hours. The 48-hour urinary excretion was 30 per cent. The patient experienced nausea for several days but no vomiting. There was a feeling of tightness in the chest and a cough productive of whitish sputum. There was a moderate depression of all of the formed elements of the blood. One month later, x-ray examination showed slight regression of the pulmonary metastases. The patient was discharged feeling well. In the ensuing weeks she appeared slightly hypothyroid, and was given desiccated thyroid in doses up to 120 mg. a day.

Upon x-ray examination on December 22, 1953, the chest appeared normal; the previously noted nodularities had disappeared. The patient complained of shortness of breath and a feeling of substernal pressure after exertion. Within two weeks, she became dyspneic at rest and acutely short of breath after walking a few steps. On January 14, 1954, a marked increase in the size of the pulmonary nodules was noted in a roentgenogram of the chest, but upon physical examination the chest was entirely normal. The

patient was admitted to the hospital on January 20, 1954, in acute respiratory distress with moderate cyanosis. The pulse rate was 104 (regular), blood pressure 110/70 mm. Hg, and temperature 99.7° F. Resonance was slightly diminished at the posterior base of the right lung, and the breath sounds were impaired. Inspiratory rales were heard at both bases. There was no distention of the neck veins, enlargement of the liver, or edema of the ankles. Vital capacity was 30 per cent of normal. Hemoglobin concentration was 13.5 Gm. per 100 ml.; hematocrit 39 per cent; white blood cell count 9,800 per cu. mm. with 74 per cent polymorphonuclears, 6 per cent band cells, 2 per cent eosinophils, 18 per cent lymphocytes; and platelet count 247,800 per cu. mm. The urine was essentially normal. Stereoscopic chest films revealed a slight increase in parenchymal infiltration, especially in the right mid-lung field, suggesting a pneumonitis superimposed upon other processes. An electrocardiogram was unremarkable. Results of blood cultures were negative.

The patient was treated with oxygen by mask. Elevations of temperature to as high as 102° F. were intermittently present despite the successive institution of various antibiotics. On the sixth hospital day, cortisone (300 mg. daily) was instituted and the temperature fell promptly to normal. The character of the breathing gradually improved over a two-week period so that the patient was not dyspneic at rest. No change in vital capacity was noted, however, and she continued to be cyanotic, with dyspnea on the slightest exertion. Signs of patchy infiltration persisted despite the continued use of antimicrobials. On the thirtieth hospital day, fever recurred along with an acute increase in dyspnea, cough and cyanosis. There were several small hemoptyses. Despite all measures there was no relief of the severe air hunger. On the following day the patient lapsed into coma and died.

Autopsy. The heart weighed 290 grams and appeared essentially normal. There was clear amber fluid (50 ml.) in each pleural cavity. Mediastinal lymph nodes were present —all measuring less than 1 cm. One of these contained papillary and follicular thyroid carcinoma.

The lungs were small, rubbery, and not compressible. The left lung weighed 400 grams, the right lung 500 grams. Irregular firm tan-colored areas up to 4 cm. in size were surrounded by regions of edematous pulmonary parenchyma. Pinpoint-sized nodules seen subpleurally in the hilar regions were the only areas of tumor grossly in evidence. Microscopically, small foci of alveolar thyroid carcinoma with patches of fibrous and hyaline tissue were noted in all lobes. The tumor cells were well differentiated but with minimal colloid. Psammoma bodies were present. The remainder of the pulmonary parenchyma exhibited the changes seen in radiation penumonitis. Where the process appeared acute, the walls of the pulmonary alveoli were lined with hyaline-like membranes. The alveolar spaces contained pigment-filled phagocytes, a few lymphocytes and many macrophages with pale foamy cytoplasm. Regeneration of alveolar epithelium with exfoliation into the alveolar spaces was also noted. The alveolar walls were edematous and the interstitium was hyperplastic, with infiltrations of lipophages, eosinophils and plasma cells. Areas of chronic change were characterized by fibrosis which obliterated the alveoli or separated them by wide bands of fibrous tissue.

Tumor metastases were not noted in any other tissues. An incidental finding was marked renal congestion and a focal acute membranous glomerulonephritis, which appeared to be recent and minimal. Small areas of central necrosis noted in the liver were attributed to anoxia. Microscopic examination of the bone marrow showed focal areas of hypoplasia.

Patient O.D. ♀

In 1943, at age 24, the patient noted a rapidly enlarging thyroid mass and several nodules in the right side of the neck. A nearly total thyroidectomy and right radical neck dissection were performed, and the pathologic diagnosis was papillary adenocarcinoma of the thyroid. Eight months later a left radical neck dissection was performed for recurrent disease; and again after five years a right cervical nodule was excised which showed papillary and follicular adenocarcinoma. Despite these procedures, no signs or symptoms of hypothyroidism developed.

Roentgenograms showed a normal chest until 1948, when numerous small nodular densities were noted in both lower lung fields. There were no respiratory complaints, however. The patient became pregnant in 1951 and a normal boy was born in July 1952. In October 1952 a radioiodine tracer study revealed a 48-hour uptake of 22 per cent over the neck, and a definite uptake in the chest. The next month she received 76 mc. of I^{131} for the purpose of completing the thyroidectomy prior to treatment of the pulmonary metastases. Thiouracil (600 mg. daily) was given for several months beginning in March 1953, and resulted in a marked increase in the uptake of radioiodine in the lung fields. The patient remained well, with no respiratory complaints and no symptoms of hypo- or hyperthyroidism.

On June 6, 1953, she was admitted for radioiodine treatment. She appeared in good health and in no distress. There was no evidence of disease in the neck. The lungs were clear to percussion and auscultation and the remainder of the physical examination was unremarkable. The serum cholesterol level was 222 mg. per 100 ml. The BMR was −31 per cent. The concentrations of blood urea nitrogen, alkaline phosphatase, and serum calcium were all within normal limits. An increase in the size and number of the metastatic nodules was noted in a roentgenogram of the chest (Fig. 2a). On June 11, 1953, the patient received 212 mc. of I^{131}, of which 16 per cent was excreted in the urine in two days. The 24-hour uptake over the neck was 8 per cent. Nausea and vomiting occurred on the second day following administration of the isotope, and promptly subsided. There was a marked depression of lymphocytes and eosinophils in the peripheral blood, but the other formed elements were essentially unaffected. She was discharged on July 3, 1953 feeling very well.

When seen as an out-patient two months later, the patient complained of coldness, fatigue, shortness of breath, and a slight nonproductive cough. She appeared moderately myxedematous. The chest was resonant throughout, but there was decreased expansion. Dry rales were heard at both lower bases. The serum cholesterol level was 296 mg. per 100 ml. A roentgenogram of the chest (Fig. 2b) showed a further increase in the size and number of the metastases. In a radioiodine tracer study 84 per cent of the administered dose was excreted in the urine within forty-eight hours, and there was minimal uptake in the chest. Triiodothyronine (70 micrograms daily) was started, but the dosage was promptly reduced to 25 micrograms daily because of complaints of nervousness and palpitation.

On the morning of September 27, 1953, an acute attack of orthopnea occurred, without fever, cough or chest pain. The patient was admitted to the hospital several hours later, semi-comatose and cyanotic. The pulse rate was 140 (regular), respirations 40, blood pressure unobtainable, and temperature 103° F. The neck veins were distended. Inspiratory rales were heard at the pulmonary bases, especially on the right. The liver was felt 2 cm. below the right costal margin. There was no peripheral edema. Hemoglobin concentration was 12.5 Gm. per 100 ml., white blood cell count 18,500, and hematocrit

37 per cent. The level of blood urea nitrogen was 22.4 mg. per 100 ml., chloride 104 mEq. per L., and CO_2 combining power 17 mEq. per L. A roentgenogram of the chest revealed bilateral pleural effusion with patchy densities in the lung fields, suggesting lower lobe bronchopneumonia. The electrocardiogram showed changes indicative of myocardial disease, consistent with acute cor pulmonale.

The patient received oxygen by mask. The blood pressure was maintained at aboue 100/80 mm. Hg with noradrenaline, and later with a transfusion of whole blood. Sht was digitalized intravenously and also received aminophylline, cortisone and penicillin. Despite these measures, respiratory distress increased and she died about sixteen hours after the onset of the acute episode.

Autopsy. The heart weighed 300 grams and appeared normal, except for moderate dilatation of the right side without hypertrophy.

There was yellowish fluid (150 ml.) in each pleural cavity. Hilar nodes were present, measuring up to 1.5 cm. in diameter. Tumor tissue was noted in one right hilar node.

The left lung weighed 600 grams and the right, 800 grams. Small tumor nodules, 3 to 7 mm. in diameter, were present beneath the translucent pleura. The lungs contained almost no air, except for a depth of 1 cm. in the upper lobes. The lower lobes were particularly firm and fibrotic. On section they were filled with hard white tumor nodules about 5 cm. in diameter, some of which had a necrotic center. The surrounding parenchyma was brown and appeared retracted, but without evidence of necrosis. The middle and upper lobes had the appearance of lobar pneumonia—red, glassy, and bulging with large amounts of hemorrhagic fluid streaming from the cut surfaces. Tumor nodules were not present in the upper lobes, except subpleurally. Microscopically, no acinar or colloid formation was seen in the tumor nodules. Tumor invasion of blood vessels was not noted. The alveolar septa were thickened and the alveolar spaces were filled with strongly eosinophilic edema fluid and large phagocytes. There were foci of fibrinoid change. In some areas single alveoli or confluent groups exhibited organization and the atypical fibroblasts that are associated with radiation effects. In addition, there were occasional foci of round cells, which suggested a superimposed focal pneumonia. E. coli was cultured from the pulmonary parenchyma. The changes in the lungs were thought to be far more diffuse than those produced by reaction to tumor, but very similar to the pneumonitis produced by x-rays. The terminal E. coli pneumonitis was thought to be of minor significance.

Microscopically, small nodules of papillary adenocarcinoma were detected in the paratracheal region of the neck; some of these appeared to lie in plugged lymphatics. In the kidneys there was some medullary congestion; in each, a single 3-mm. yellow nodule was present, which was shown microscopically to be tumor. There was central congestion of the liver. The remaining organs, including the bone marrow, were unremarkable.

CLINICAL PROGRESS IN THE TREATMENT OF EXOPHTHALMOS OF GRAVES' DISEASE, WITH PARTICULAR REFERENCE TO THE EFFECT OF PITUITARY SURGERY*

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THE two chief manifestations of Graves' disease are hyperplastic goiter with hyperthyroidism and exophthalmos, the onsets of which usually are simultaneous. However, exophthalmos sometimes is the only manifestation of the disease. We consider Graves' disease to be the result of thyroid-pituitary dysfunction.

Exophthalmos that occurs in patients having Graves' disease is characterized not only by proptosis but also by various associated changes. These changes include retraction of the eyelids and manifestations resulting from increase in bulk of periocular tissues. This increase in bulk is caused by increases in the amount of fat and of mucopolysaccharides, and by an edema that has been proved not to be associated with an excess of water in the tissues (1, 2). The extraocular muscles become enlarged and infiltrated with fat and lymphocytes; eventually they become paralyzed and fibrosed. The proptosis may be extreme, with slight swelling of the lids and conjunctiva. Troublesome symptoms usually include a burning sensation of the eyes, pain, lacrimation, and diplopia (most commonly on upward gaze).

After control of the hyperthyroidism, exophthalmos subsides in almost 60 per cent of the patients; however, it fails to improve or becomes worse in about 40 per cent, and becomes extremely severe in about 1 per cent. Exophthalmos is believed to be due to hypersecretion of thyroid-stimulating hormone or of a closely related material called *exophthalmos-producing substance* (3, 4). Attempts at reducing exophthalmos apart from orbital decompression have been directed largely at suppression of pituitary hyperactivity. In many instances the pituitary hyperactivity, and with it the exophthalmos, gradually improves spontaneously. For this reason, before a specific treatment can be classified as successful, the improvement that follows its use must be significant in amount and prompt in a large

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number of patients. By these criteria, treatment with iodine, estrogen or androgens generally has been unsuccessful.

It is the purpose of this paper to discuss briefly the present status of roentgen therapy, and the use of thyroid hormones, corticotropin (ACTH) or cortisone in the treatment of the exophthalmos of Graves' disease, and to discuss in more detail our results in 9 patients having exophthalmos treated by hypophyseal surgery.

ROENTGEN THERAPY

Roentgen irradiation to the pituitary region, usually in doses of 2,000 to 4,000 r at the surface, appears to us to be of doubtful value in the treatment of exophthalmos. Improvement after treatment has been reported as none, slight, or variable. A decrease in proptosis of more than 3 mm. rarely has occurred; and when it has, it has been very gradual. Our confidence in this method of treatment is reduced by the following facts: First, doses of roentgen irradiation as high as 10,000 r have not produced measurable hypopituitarism (5). Secondly, the effects of irradiation as determined by assay of other pituitary hormones (6) or by sperm counts (7) have been evanescent (Fig. 1), although in some patients exophthalmos continues to improve. Thirdly, the improvement that does occur often is relatively insignificant, the recession of proptosis amounting to less than 1.5 mm. Finally, improvement occurring months or even years after irradiation has been claimed to be the result of this treatment. Lamberg (8) has recently published an excellent review of the results of roentgen therapy.

ADMINISTRATION OF DESICCATED THYROID, THYROXINE, OR TRIIODOTHYRONINE

In the past it has been common practice to give enough desiccated thyroid or thyroxine to patients with exophthalmos to prevent hypothyroidism or even to prevent hypometabolism not clearly hypothyroid. This procedure has been followed in the hope of preventing or decreasing existing hyperpituitarism. In some cases, doses of desiccated thyroid or of thyroxine in larger than physiologic amounts have been administered in an

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FIG. 1. Pronounced swelling of orbital soft tissues in a 69-year-old woman who had mild diabetes but no evidence of hyperthyroidism.

FIG. 2. Same patient, one week later and prior to treatment. Note rapidity of progression of the changes.

FIG. 3. Same patient, after three weeks of therapy with *l*-triiodothyronine, 100 μg. per day.

FIG. 4. Same patient, four months after beginning of *l*-triiodothyronine therapy, showing marked diminution of soft-tissue swelling. Vision was greatly improved.

attempt to reduce exophthalmos, but the improvement usually has been clinically insignificant, or temporary. There are no data to suggest that triiodothyronine produces better results than could be produced by desiccated thyroid or thyroxine used in sufficient doses or for sufficient periods of time.

We have made repeated observations on 20 patients with progressive exophthalmos treated with triiodothyronine over periods ranging from two to eleven months. During this time, proptosis was reduced by 2 mm. or more in 9 patients; it remained stationary in 9; and became worse in 2. When there was significant recession, it was usually evident at the first visit, two months after beginning treatment. It should be borne in mind that in those patients in whom there was no recession there had been a steady increase in the exophthalmos before treatment, and it appeared that this increase may have been halted by the therapy.

The most striking changes were seen in a 69-year-old woman in whom there was no evidence of, or history of hyperthyroidism (Fig. 1). She was blind and had mild diabetes mellitus. There was extreme soft-tissue swelling and proptosis, both of which were rapidly becoming worse (Fig. 2). After beginning treatment with triiodothyronine improvement was evident in a few days and was pronounced in three weeks (Fig. 3). Later the swelling of the lids and conjunctiva almost completely disappeared, there was a recession in proptosis (5 mm., left eye; 6 mm., right eye), and vision progressed from total blindness to fairly good eyesight (Fig. 4). Ninety per cent of these changes occurred within three months after treatment had been started. Further details of the improvement seen in other patients in this series are being published elsewhere (9).

ADMINISTRATION OF CORTICOTROPIN, WITH OR WITHOUT CORTISONE OR HYDROCORTISONE

Since 1950, various attempts have been made to treat exophthalmos locally or systemically with corticotropin (ACTH) alone or combined with cortisone or hydrocortisone (10). Early trials were not very promising, but in 1953 Kinsell (11) reported encouraging results in 9 patients having malignant exophthalmos who were treated with large doses of ACTH and cortisone; some of them were followed for as long as two years. Improvement occurred in all 9 patients, but in 2 there were serious complications—temporary psychosis in one and perforated peptic ulcer in another. Recently reported (12) from England were the results in 28 patients having exophthalmos, 5 of whom received ACTH and 23 of whom received cortisone, mostly in relatively small doses. In 8 of these patients there was moderate-to-marked improvement.

We have treated 10 patients having extremely severe progressive exoph-

FIG. 5. Severe progressive exophthalmos and pronounced soft-tissue infiltration in a patient with Graves' disease.

FIG. 6. Same patient, two weeks after beginning of treatment with corticotropin (ACTH) for five days, and hydrocortisone subsequently.

thalmos with ACTH in a dosage of 40 mg. administered intravenously over a period of six hours daily for five to sixteen days, and hydrocortisone in an initial dosage of 200 mg. orally daily and diminished over many weeks. Improvement occurred in all 10 patients. In 1 of these patients (Fig. 5) a dramatic diminution of extensive soft-tissue swelling and a 2-mm. reduction in proptosis occurred within a few days after the beginning of treatment (Fig. 6). In this patient and in some of the others, active hyperthyroidism disappeared in a few days, and the thyroidal I^{131} uptake, serum protein-bound iodine concentration, basal metabolic rate and serum cholesterol level became nearly or completely normal.

PITUITARY SURGERY

In 1937, two of us (E.P.M., W.J.G.) (7) observed that cauterization of the pituitary sometimes is followed by disappearance of exophthalmos. However, the severity of the induced hypopituitarism could not be limited at will; and at that time, if severe pituitary failure occurred, it was not possible to treat it adequately because cortisone was not yet available.

Recently the operation has been revived and modified. The procedure always is reserved for cases in which nonsurgical methods have failed and the condition is extremely severe. Nine patients so far have been treated

by section of the pituitary stalk or cauterization of the anterior lobe, or both. In all 9 patients the exophthalmos improved postoperatively; in some it disappeared almost entirely, and visual acuity that had been extremely poor returned to normal. In recent cases, when section of the stalk is performed, an impervious diaphragm of waxed paper, plastic or tantalum is placed above the sella turcica to prevent re-establishment of the portal circulation. The importance of interfering with the portal circulation of the pituitary (13), and the fact that section of the stalk is followed in rabbits by pronounced atrophy of the pituitary and diminution in thyroid and adrenal function, have been clearly demonstrated by Harris and co-workers (14–16). Section of the stalk without anterior lobe cauterization has been performed in only 2 patients. So far as we know, the only other report of a case in which malignant exophthalmos was treated by pituitary surgery is that by Albeaux-Fernet and associates in 1955 (17).

The reports on 2 of our 9 patients (Cases 1 and 2)—1 treated by pituitary cauterization and section of the stalk, and 1 treated by section of the stalk alone—are presented in detail in order to compare the results of the two types of treatment. Findings in the remaining 7 patients are summarized in Table 1. Four of these 7 patients underwent unilateral orbital decompression in addition to pituitary surgery.

Case 1

A 25-year-old woman was examined on July 2, 1954, because of diminution in vision during the preceding five weeks and a corneal ulcer of five days' duration. She had hyperthyroidism of one and a half years' duration that had been diagnosed and treated elsewhere. Six months after the onset of hyperthyroidism and two weeks after the death of the patient's mother, exophthalmos had first been evident. Hyperthyroidism had been controlled by the administration of iodine and propylthiouracil for six months and propylthiouracil without iodine for three months. After the onset of hyperthyroidism she had lost 25 pounds in weight, some of which had been regained.

On physical examination, there was a diffuse goiter estimated to weigh approximately 40 grams, a pulse rate of 100 per minute, a fine tremor, and cutaneous changes characteristic of hyperthyroidism. The blood pressure was 124/74 mm. Hg. Also noted were severe infiltrative exophthalmos, extreme lid retraction, severe multiple extraocular muscle palsies, a left corneal ulcer, and proptosis. The exophthalmometric measurements were 30 mm. right, and 31 mm. left. Results of laboratory tests were normal, including a basal metabolic rate of −5 per cent, a 24-hour thyroidal radioactive iodine uptake of 33 per cent, and a serum cholesterol concentration of 230 mg. per 100 ml.

During the first ten days in the hospital, there was a definite increase in chemosis and exophthalmos bilaterally, with further limitation of gaze in all directions.

A right frontal craniotomy was performed and the anterior lobe of the pituitary was treated with electrocautery. The stalk was thoroughly cauterized where it entered the hypophysis, and it was divided. A piece of waxed paper was placed between the divided ends of the stalk.

Five days postoperatively, improvement was observed in the chemosis and edema of the lids (Fig. 7). For at least two weeks there was a steady and dramatic improvement

TABLE 1. SUMMARY OF FINDINGS IN 7 PATIENTS HAVING MALIGNANT EXOPHTHALMOS TREATED BY HYPOPHYSEAL OPERATION

Patient			Date	Exophthalmometric measurements (mm.)		Remarks
Case No.	Age (yrs.)	Sex		OD	OS	
3	53	F	Dec. 19, '36	23	24	
			May 17, '37	Severe, but not measured		Right orbital decompression, section of pituitary stalk, and pituitary cauterization.
			June 16, '37	—	—	Definite improvement.
			June 20, '37	25	23	
			Feb. 14, '38	22	19	
			Nov. 30, '38	19	18	
			1946	—	—	Died of other causes.
4	49	M	Oct. 25, '39	33	35+	
			Oct. 27, '39	—	—	Left orbital decompression, section of pituitary stalk, and pituitary cauterization.
			Nov. 15, '39	30	32	
			Dec. 4, '39	—	—	Orbital swelling less; visual acuity improved.
			May 15, '44	30	30	
			Jan. 9, '45	29	28	
			Apr. 22, '53	29	28	
			1954	—	—	Died of vascular accident.
5	52	M	Sept. 9, '40	28	28	
			Sept. 10, '40	—	—	Section of pituitary stalk and pituitary cauterization.
			Sept. 11, '40	—	—	Improvement in exophthalmos.
			Sept. 13, '40	—	—	Died of an intracranial hemorrhage.
6	58	M	Nov. 19, '41	29	30	
			Dec. 9, '41	—	—	Left orbital decompression, section of pituitary stalk, and pituitary cauterization.
			Apr. 27, '42	29	25	Hypopituitarism
			June 17, '42	30	25	
			Feb. 11, '43	28	25	
			Nov. 6, '44	24	23	
			July 1946	—	—	Died of acute pulmonary edema and chronic glomerulonephritis.

TABLE 1—(*continued*)

Patient			Date	Exophthalmometric measurements (mm.)		Remarks
Case No.	Age (yrs.)	Sex		OD	OS	
7	61	M	Jan. 5, '46	31.5	29	
			Jan. 7, '46	—	—	Left orbital decompression and pituitary cauterization.
			Jan. 19, '46	26	24	
			May 22, '46	29	24	
			Oct. 1953	—	—	Died of an intracranial hemorrhage.
8	64	M	Sept. 20, '53	27	25	
			Sept. 25, '53	—	—	Bilateral orbital decompression; improvement in exophthalmos for six weeks, then more severe than preoperatively.
			Dec. 9, '53	—	—	Section of pituitary stalk and anterior pituitary cauterization.
			Dec. 23, '53	—	—	Swelling decreased 50 per cent; marked improvement in visual acuity.
			Dec. 30, '53	—	—	Left colectomy for gangrene of bowel due to thrombosis.
			Jan. 2, '54	—	—	Died of massive multiple pulmonary emboli.
9	58	M	Sept. 7, '56	28·29	25·26	I^{131}, 4.0 mc.
			Oct. 31, '56	—	—	Marked conjunctival chemosis.
			Nov. 28, '56	—	—	Section of pituitary stalk; hydrocortisone, 10 mg. three times daily, started.
			Dec. 20, '56	—	—	Enucleation, left eye; I^{131}, 6.0 mc.
			Jan. 14, '57	—	—	Chemosis subsiding.
			Feb. 12, '57	—	—	Further reduction in chemosis.
			Apr. 23, '57	—	—	Conjunctival chemosis less marked.

in the exophthalmos: most of the lid swelling and much of the chemosis disappeared. During this time there was no evidence of hyperthyroidism.

Results of laboratory tests performed nine days after operation (July 21) were as follows: urinary gonadotropin less than 6 mouse units per twenty-four hours (18);

17-hydroxycorticoids (19), 4.7 mg., and 17-ketosteroids (20), 3.7 mg. per twenty-four hours; and a 58 per cent decrease in total circulating eosinophils during an ACTH test. Three weeks after operation, a urinary-tract infection developed that was successfully treated with sulfa drugs and penicillin. About two weeks postoperatively, the patient had excessive thirst and polyuria, but these symptoms were alleviated by the administration of Pitressin.

One month postoperatively (August 3), the patient's weight was 15 pounds less than it had been before operation. Physical examination revealed a warm, moist skin and a tachycardia of 110 per minute. At this time the basal metabolic rate was +19 per cent and a thyroidal 24-hour I^{131} uptake was 95 per cent. A most remarkable change had occurred: she had a recurrence of active hyperthyroidism in spite of the pituitary operation; and during this exacerbation, the ocular signs became almost as severe as they had been before the operation (Fig. 8). Again it was feared that she might lose one or both eyes, and orbital decompression was considered. The hyperthyroidism was controlled by the administration of Lugol's solution for two weeks, and then propylthiouracil. During this therapy, the basal metabolic rate decreased to −43 per cent and the proptosis again increased markedly, but without associated periorbital edema (Fig. 9).

Two months after operation (September 14) the basal metabolic rate was −43 per cent and a thyroidal 24-hour I^{131} uptake was 88 per cent. Because of continual thirst and polyuria and the high uptake of radioactive iodine, the administration of Pitressin and propylthiouracil was continued.

Signs and symptoms of pituitary failure were pronounced five months after operation (December 14). Symptoms included ease of fatigue, nocturia and polyuria when the administration of Pitressin was temporarily stopped, amenorrhea, intolerance of cold, dry skin, and reduced body hair. In addition there were severe hot flashes that seemed typical of those during the menopause. Such hot flashes are not usually associated with pituitary failure; they disappeared when estrogen was given, and reappeared when it was stopped. The proptosis and the ocular swelling were less.

Results of laboratory tests were as follows: basal metabolic rate, −26 per cent; thyroidal 24-hour I^{131} uptake, 68 per cent; blood cholesterol concentration, 269 mg. per 100 ml.; urinary gonadotropin, less than 13 mouse units per twenty-four hours; and positive findings with the Power-Kepler water excretion test.

The patient was given replacement therapy in the form of Pitressin as required, stilbestrol cyclically, and desiccated thyroid in a dosage of ½ grain daily. The administration of propylthiouracil was continued but the dosage was decreased.

Ten months after operation (May 1955), the patient was asymptomatic. She no longer needed Pitressin, artificial menses were occurring at normal intervals, and her eyes continued to improve. She had lost 15 pounds in weight during the preceding three months. Results of laboratory tests were as follows: basal metabolic rate, −23 per cent; serum cholesterol level, 243 mg. per 100 ml.; and a 38 per cent fall in eosinophils during the ACTH test. The dosage of propylthiouracil was further reduced, and the dosage of thyroid was increased to 1 grain daily.

Sixteen months postoperatively (November 1955), her eyes continued to improve. Exophthalmometric readings at this time were 25 mm. right, and 26 mm. left. The basal metabolic rate was −24 per cent. The dosage of thyroid was increased to 1½ grains daily.

Almost twenty-one months postoperatively (March 22, 1956), the patient felt well except for some sleepiness. Axillary and pubic hair were sparse. Results of laboratory tests at this time were as follows: basal metabolic rate, −14 per cent; thyroidal 24-hour I^{131} uptake, 10 per cent; urinary 17-hydroxycorticoids, 0.6 mg., and 17-ketosteroids, 2.3 mg. in twenty-four hours. Stilbestrol therapy was continued. The administration of

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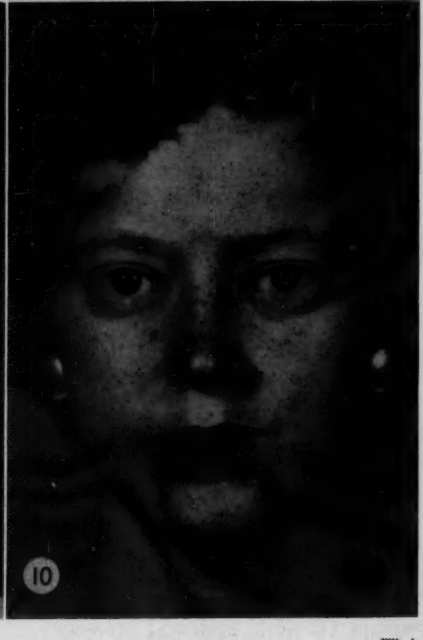
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propylthiouracil was stopped, and because of a misinterpretation of orders the patient discontinued taking desiccated thyroid as well.

When she returned five months later (August 10, 1956), she had typical signs and symptoms of myxedema with a basal metabolic rate of −39 per cent, a blood cholesterol concentration of 492 mg. per 100 ml., and a thyroidal 24-hour I^{131} uptake of 50 per cent. On assay, 24-hour urinary hormone concentrations were again found to be low—gonadotropin, less than 13 mouse units; 17-hydroxycorticoids, 0.7 mg., and 17-ketosteroids, 2.4 mg. Her eyes continued to improve. The administration of desiccated thyroid (1½ grains daily) was resumed, and hydrocortisone (10 mg. daily) was added to the regimen. After one month, desiccated thyroid was stopped for two weeks, after which the serum protein-bound iodine (PBI) level was 5 μg. per 100 ml.

Stilbestrol therapy was discontinued for one month, but had to be restarted because the patient again had hot flashes and amenorrhea. A moderate hypochromic anemia occurred that responded to iron therapy.

Two and one-half years after pituitary surgery (November 23, 1956), the patient felt very well and there was only minimal residual exophthalmos (Fig. 10). Exophthalmometric readings were 24 mm. and 23 mm. for right and left eyes, respectively, compared to 32 mm. in each eye when the exophthalmos had been at its peak. The dosage of desiccated thyroid was increased to 3 grains daily in an attempt to relieve the hypothyroidism further. The basal metabolic rate was −25 per cent and the blood cholesterol concentration was 300 mg. per 100 ml. There was a suggestion of a return of the polydipsia and polyuria after hydrocortisone was started.

Current therapy in this patient consists of desiccated thyroid, 3 grains daily; stilbestrol cyclically, 1 mg. daily; and hydrocortisone, 10 mg. daily. After she had followed this regimen for three months, the basal metabolic rate was −15 per cent, serum cholesterol level 284 mg. per 100 ml., and thyroidal I^{131} uptake 4 per cent in twenty-four hours.

Comments on Case 1. Some of the most remarkable features of the course of this patient were as follows:

1. The prompt appearance of the exophthalmos following a serious emotional disturbance.

2. The rapid and pronounced reduction in infiltrative ocular phenomena within the first two weeks after pituitary cauterization.

3. The reappearance of hyperthyroidism about one month after pitui-

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FIG. 7. *Case 1.* Primary improvement of exophthalmos of Graves' disease in a 25-year-old woman, eight days after section of the stalk and pituitary cauterization. A preoperative photograph was not obtained, but before operation her appearance resembled that in Figure 8.

FIG. 8. *Case 1.* Three and a half weeks after pituitary operation. Exacerbation of ocular signs to the preoperative degree of severity was associated with reappearance of the clinical signs of hyperthyroidism.

FIG. 9. *Case 1.* Two months after pituitary operation. During propylthiouracil therapy, the basal metabolic rate decreased to −43 per cent and proptosis increased without associated periorbital edema.

FIG. 10. *Case 1.* Two and one-half years after pituitary operation. Note disappearance of all but minimal exophthalmos. Patient was asymptomatic and was receiving supportive therapy.

tary surgery, associated with a definite increase in the swelling of the eyelids and conjunctivae, and a decrease in these manifestations during control of the hyperthyroidism with iodine and propylthiouracil.

4. The exacerbation of the proptosis without an increase in infiltrative phenomena during suppression of the basal metabolic rate to −43 per cent.

5. The appearance of hot flashes, like those of the menopause, during increased pituitary failure; these were controlled by administration of estrogen and recurred when it was withdrawn.

6. The occurrence of many features of hypopituitarism, including a low level of urinary gonadotropin and steroids, amenorrhea, loss of axillary and pubic hair, and the appearance of full-blown myxedema after inadvertent interruption of thyroid therapy.

7. The increase in the thyroidal uptake of I^{131} from low levels to 50 per cent after the cessation of thyroid therapy, in the presence of hypopituitarism and clinical myxedema. We were disinclined to believe this estimate of I^{131} uptake but the test was repeated several times with similar results.

8. The gradual and complete disappearance of all evidence of ocular signs of Graves' disease and return of vision to normal. Visual acuity had been as low as 6/21 in the right eye and 2/60 in the left eye, and became 6/6 in each eye eight months after treatment. There was complete recovery of movements of extraocular muscles.

9. The patient now is symptom-free and in good health; she still is taking desiccated thyroid, stilbestrol and hydrocortisone.

Case 2

A 41-year-old woman had first noticed typical signs of hyperthyroidism and exophthalmos eight months before initial examination here. A little later, diplopia with associated palsies of the extraocular muscles and retraction and pronounced edema of the lids developed. Although she appeared clinically to have mild hyperthyroidism, the results of tests were equivocal. She had lost 11 pounds in weight since the onset of hyperthyroidism. The menstrual periods were normal. On initial examination (March 4, 1956), the basal metabolic rate was +19 per cent, the 24-hour thyroidal I^{131} uptake was 28 per cent, the level of serum PBI was 6 μg. per 100 ml., and serum cholesterol concentration 140 mg. per 100 ml. After administration of 4 units of thyrotropin, the 24-hour thyroidal I^{131} uptake increased to 63 per cent and was not diminished by triiodothyronine or desiccated thyroid, being 68 per cent after the patient had received 100 micrograms of *l*-triiodothyronine daily for ten days, and 77 per cent after administration of 4 grains of desiccated thyroid daily for ten days.

For four months after the initial examination, the exophthalmos steadily became worse and the proptosis increased from 24 mm. and 23.5 mm. to 25 mm. bilaterally, during which time the patient had received 100 micrograms of *l*-triiodothyronine daily for twenty-eight days and 200 mg. of propylthiouracil daily for fourteen days. Hypophyseal operation was advised. Preoperative 24-hour values for urinary 17-ketosteroids were 9.4 and 8.1 mg., and for 17-hydroxycorticoids 1.8 and 4.8 mg., on May 26 and July 3 respectively.

On July 5, 1956, a craniotomy was performed, the pituitary stalk was severed, and a plastic diaphragm was placed above the sella turcica. After operation, mild diabetes insipidus developed. Two months postoperatively results of the Carter-Robbins test were mildly positive.

One and a half weeks after operation (July 16) the periocular swelling had diminished. Exophthalmometric measurements were 24 mm. and 23 mm. in the right and left eyes, respectively. The thyroidal I^{131} uptake remained relatively high, being 56 per cent in twenty-four hours.

In September, 1956, two and a half months postoperatively, she was amenorrheic, the cells in the vaginal smear resembled those of the castrate, and the endometrium was atrophic. The basal metabolic rate was +18 per cent, and the serum PBI level was 7 μg. per 100 ml. The urinary gonadotropin titers were less than 13 mouse units per twenty-four hours on each of two occasions. Repeated measurements of serum potassium and sodium indicated normal concentrations. The 24-hour urinary excretions of 17-ketosteroids (4.4 mg.) and of 17-hydroxycorticoids (2.2 mg.) were not decidedly abnormal, but they were lower than they had been before operation. Five months (December 6, 1956) and seven months (February 22, 1957) postoperatively the serum PBI levels were 3 μg. and 2 μg. per 100 ml. respectively, and the basal metabolic rates were +5 and +6 per cent respectively. Clinical evidence of hyperthyroidism had disappeared without other treatment than the pituitary surgery. Pitressin and small doses of cortisone given postoperatively were believed to have had insignificant effects.

The severity of the proptosis seven months after operation (February 22, 1957) was similar to that before operation—25 mm. bilaterally. However, before operation there had been a progressive increase in all of the associated ocular signs, whereas after operation most of these improved and none increased. In other ways the patient's eyes also were decidedly better. There was a definite lessening of swelling of the eyelids and the diplopia that had existed preoperatively had disappeared, although careful testing showed residual muscle weakness. She could now read newsprint that she could not read preoperatively.

Ten months postoperatively (May 15, 1957), definite evidence of hypopituitarism had developed. Symptoms included fatigue, amenorrhea, intolerance to cold, dry skin, and reduced body hair. The swelling of the eyelids had practically disappeared and visual acuity continued to improve. The proptosis was less, exophthalmometric measurements being 23 mm. and 22 mm. in the right and left eyes, respectively. Results of laboratory tests were as follows: basal metabolic rate, −17 per cent; 24-hour thyroidal I^{131} uptake, 34 per cent; and serum cholesterol concentration, 203 mg. per 100 ml. The administration of thyroid, ½ grain daily, was started at this time.

Comments on Case 2. Among the interesting features of the course of the disease in this patient were:

1. Section of the pituitary stalk and placing of a polyethylene diaphragm above the sella turcica was followed by measurable but not severe hypopituitarism.

2. The hypopituitarism was manifested by amenorrhea, low urinary gonadotropin, atrophic endometrium, low basal metabolic rate, and abnormally low serum PBI values; thus, both gonadotropin and thyrotropin apparently were affected.

3. The clinical evidence of mild hyperthyroidism disappeared and the

basal metabolic rate decreased from a slightly elevated level to below the normal range.

4. Exophthalmos that had been increasing in severity changed its course. The proptosis decreased and other signs, including swelling of the eyelids, muscle palsy, and visual acuity improved.

DISCUSSION

From our limited experience it appears that some degree of hypopituitarism can be produced by section of the pituitary stalk. Following cauterization of the anterior hypophysis and severing of the stalk, the hypopituitarism that develops is both more rapid and more severe than that which follows section of the stalk alone. Judging from these cases it appears that the more severe the pituitary failure the more rapid and more complete is the recession or disappearance of the ocular signs of Graves' disease.

Two of the patients who underwent section of the pituitary stalk accomplished by cauterization of the anterior lobe died during the immediate postoperative period. The death of one (Case 5) of these 2 patients was clearly the result of an operative accident and need not necessarily have any direct bearing in the future on deaths connected with this procedure. The death of the other patient (Case 8) was surrounded by most unusual circumstances. This woman had arterial hypertension and severe, long-standing diabetes mellitus; we think it reasonable to assume that vascular damage due to the diabetes and hypertensive cardiovascular disease were important factors in the complication of vascular accidents. She apparently was recovering satisfactorily after colectomy necessitated by mesenteric thrombosis—she was up and about the room and the eye signs were continuing to improve—until massive multiple pulmonary emboli occurred followed by sudden death.

Additional observations are clearly needed, and it will be particularly interesting to learn what degrees of hypopituitarism and of improvement in ocular signs will consistently follow section of the pituitary stalk—which is a relatively nontraumatic procedure compared to cauterization of the pituitary, or to hypophysectomy. Although the rate or degree of improvement in exophthalmos after section of the stalk alone may not be so great as that after more radical hypophyseal operations, section of the stalk may prove to be an extremely useful procedure in many patients having severe exophthalmos. If section of the pituitary stalk is proved to produce consistently good results, it may obviate the necessity of orbital decompression in some instances. However, in cases of exophthalmos in which there is great urgency and serious danger of loss of the eyes without

immediately effective treatment, orbital decompression is perhaps the procedure of choice.

SUMMARY

The results of treatment of 39 patients with severe exophthalmos are presented. Twenty patients were treated with large doses of triiodothyronine. Proptosis was reduced by 2 mm. or more in 9, remained stationary in 9, and became worse in 2. Ten patients were treated with ACTH and hydrocortisone in diminishing doses for varying periods of time. Improvement occurred in all. Exophthalmos also improved in 9 patients who underwent pituitary surgery, but 2 of these cases terminated fatally. In comparing the results of a combination of pituitary cauterization and section of the stalk with those of section of the stalk alone plus insertion of a suprasellar diaphragm, it was found that the former procedure caused more rapid and severe hypopituitarism with more rapid improvement in the exophthalmos. However, section of the stalk plus insertion of a diaphragm without direct attack upon the anterior lobe was followed by hypopituitarism.

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AUTO-IMMUNITY IN HASHIMOTO'S DISEASE AND ITS IMPLICATIONS*

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RECENT workers (1, 2, 3) have described abnormalities in the serum proteins of patients with Hashimoto's disease, namely, high values for gamma globulins and for the results of flocculation tests. The highest values were obtained in untreated patients with large goiters. During the months following removal of the goiter, these abnormal levels slowly returned to the normal range (4).

The known association of gamma globulins with circulating antibodies and the infiltration of the diseased thyroid gland with plasma cells and lymphoid tissue (which are known to produce antibodies) suggested that the disease process and the postoperative findings might be explained if it were postulated that these patients were immunized against an antigen in the thyroid gland. Confirmation of this hypothesis is provided by the results of the present studies, which demonstrate the presence of thyroid-specific precipitating auto-antibodies in the sera of such patients. Preliminary reports of this work have appeared (5, 6).

MATERIALS AND METHODS

The antigen was prepared from thyroid glands obtained at operation or at autopsy from either thyrotoxic or normal subjects. The glands were thinly sliced and allowed to stand with three parts of 0.9 per cent saline at 2° C. overnight. The extract was then centrifuged and the supernatant liquid stored in a deep-freeze until required. For the test, this extract was diluted threefold with saline. Purified thyroglobulin was prepared from the extract by the method of Derrien, Michel and Roche (7). New Zealand agar (1 per cent) in physiologic saline was used, with sodium azide (1 per cent) added as an antibacterial agent. The agar was clarified by the method of Feinberg (8).

The precipitin tests were made on fresh serum which, if necessary, was centrifuged at 13,000 rpm until clear. In early experiments the antigen solution was layered directly onto the serum, but diffusion-precipitation in agar gels was found to be more sensitive and has been used in the studies reported here. The procedure was adapted from Oakley and Fulthorpe's (9) modification of the Oudin (10) method. The patient's serum was mixed with an equal volume of agar at 42° C. and pipetted into a 9.0×0.5 cm. tube. When the mixture had set, a second layer of 0.5 per cent saline agar was added, followed by a final layer of the thyroidal saline extract mixed with an equal volume of 1 per cent agar. The tubes were left either at 2° or at 22° C., but sudden changes of temperature were avoided. A precipitation zone appeared in the saline layer after one to ten days.

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Gradual movement of the leading edge of a precipitation zone towards the serum layer occurred with weak precipitins.

Electrophoretic separation of serum proteins (11) and estimations of thymol turbidity (12), colloidal-gold flocculation (13) and zinc-sulphate turbidity (14) were carried out in the routine laboratory of the Courtauld Institute of Biochemistry. Total serum protein levels were estimated by a biuret method and gamma globulin values were calculated from the scanned electrophoretic strips dyed with Azocarmin B, as described previously (4). Results obtained by this technique are higher than those obtained with methods based upon boundary electrophoresis, but are useful for comparative studies.

For more detailed studies, serum gamma globulins were separated by zone electrophoresis on cellulose columns (15, 16) under conditions described in a previous note (6).

RESULTS

In 25 of 30 patients with Hashimoto's disease, the serum contained precipitins against saline-extracted human thyroid gland (Table 1). The thyroid specificity of the antibody was proved by the negative results obtained when the sera were tested against saline extracts of human liver, kidney, spleen, lymph nodes, parotid gland and brain. Similar precipitin reactions were observed when purified thyroglobulin was substituted for the crude saline extracts of human thyroid. Thyroglobulin was identified as the active antigen. This was shown by the confluence of the precipitin lines when the crude thyroid extract and purified thyroglobulin reacted with Hashimoto serum in the gel diffusion technique of Ouchterlony (17) (Fig. 1). Fractionation of Hashimoto serum by zone electrophoresis, followed by precipitin tests on the eluted fractions, showed that the antibody was located exclusively in the gamma globulins. This was confirmed by the technique of immunoelectrophoresis in agar (18).

In strongly-reacting sera examined by the modified Oudin technique, a compact precipitation zone appeared after sixteen to forty-eight hours and remained stationary when antibody and antigen were present in equivalent proportions (19). In weakly-reacting sera the precipitation bands were more diffuse, took up to two weeks to become clearly visible, and gradually moved downwards as the precipitate redissolved in excess antigen. These effects are illustrated in Figure 2, which shows a precipitin test performed on serial dilutions of a Hashimoto serum containing a high titer of antibody. Frequently 2 precipitin lines were observed even when purified thyroglobulin was used, indicating the presence of two distinct antigenic components.

Previous work (20, 21) had shown that anti-human-thyroglobulin sera obtained from rabbits cross-reacted to a small extent with thyroglobulin from a variety of mammalian species. The sera of Hashimoto patients gave no cross-reactions when tested against extracts of thyroid from the rabbit, rat, sheep, hog, cow and horse.

Table 1 shows the thyroglobulin precipitin reactions, results of floccu-

Center well in Ouchterlony plate contains serum from patient with Hashimoto's disease. In counterclockwise direction, beginning at the top, the others contain:

1. Purified thyroglobulin (5 mg./ml.).
2. Extract of thyroid (1:10).
3. Purified thyroglobulin (10 mg./ml.).
4. Extract of thyroid (1:20).

FIG. 1: Identification of thyroglobulin as the active antigen in the saline extract of thyroid using the diffusion-precipitation technique in agar gel (16). The confluence of the precipitation lines at the corners shows the identity of the antigens in the wells containing the crude extract of thyroid and pure thyroglobulin.

lation tests and serum gamma globulin values in 30 patients with Hashimoto's disease. In the 11 untreated patients, values for flocculation tests and gamma globulins were raised above normal, and strong precipitins were found in 7 cases. In 2 untreated patients the precipitins were weak despite a marked elevation of the flocculation values, and in 2 the sera failed to yield any precipitins by the technique used.

Of the 14 patients (Nos. 12–25) treated with desiccated thyroid in doses

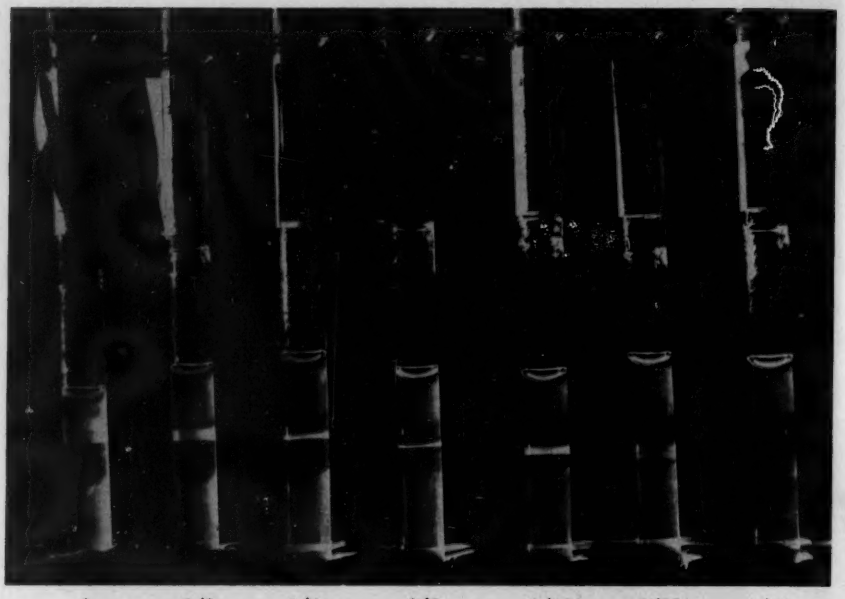

FIG. 2: Thyroglobulin precipitin test performed on serial dilutions of serum from a patient with Hashimoto's disease, using a single antigen concentration. Precipitins were visible to 1/64 dilutions of the serum. The compact band seen in 1/8 dilution indicates equivalence of antigen and antibody. The leading edge of the precipitate approaches the serum layer with progressive dilution. Photographed after three weeks at 2° C.

of 120 to 240 mg. daily for one to three years, 7 had strong precipitins in spite of the fact that in all but 1 instance the goiters had regressed almost completely as a result of the treatment, and in some instances the results of flocculation tests and the gamma globulin levels had returned to normal from previously elevated values. In 5 of these patients the precipitins were weak, and in 2 no precipitins could be demonstrated.

The sera in 5 cases of Hashimoto's disease (Nos. 26–30) were examined within a year of partial thyroidectomy. In 3 instances the precipitin reaction was strong, in 1 it was weak, and in 1 there was no reaction though the test was made only three weeks after incomplete removal of the goiter. In Cases 26 and 27, gamma globulin levels were high before operation and gradually returned towards normal within six months of thyroidectomy; the precipitin reactions were still strong eleven and nine months respectively after operation.

It may be seen from Table 2 that the proportion of cases in which there were no precipitins increased with the amount of thyroid tissue removed at

TABLE 1. THYROGLOBULIN PRECIPITIN REACTIONS, SERUM GAMMA GLOBULIN LEVELS, AND RESULTS OF FLOCCULATION TESTS IN 30 PATIENTS WITH HASHIMOTO'S DISEASE

Patient No., Sex & Age	Treatment and duration	Flocculation tests			Gamma globulins (Gm./100 ml.)*	Thyroglobulin precipitins
		Thymol turbidity (units)*	Colloidal gold (units)*	Zinc sulphate (units)*		
1† F 47	None	8	4	17	2.4	+++
2† F 61	None	18	5	31	3.3	+++
3† F 58	None	5	1	13	2.8	+++
4† F 42	None	8	5	19	2.2	+++
5 M 47	None	20	5	40	2.8	+++
6 F 44	None	9	3	13	1.9	+++
7 F 52	None	20	5	44	3.1	+++
8† F 54	None	12	5	25	2.5	+
9† F 48	None	12	5	22	—	+
10 F 45	None	6	4	14	2.1	−
11 F 42	None	14	5	18	—	−
	Desicc. thyroid for:					
12 F 45	12 mos.	18	5	22	2.8	+++
13 M 69	2 yrs.	4	5	11	1.6	+++
14 F 66	18 mos.	9	3	11	2.3	+++
15 F 48	3 yrs.	7	2	10	2.4	+++
16 F 52	12 mos.	3	2	13	2.0	+++
17 F 45	2½ yrs.	4	1	8	1.5	+++
18† F 46	3 yrs.	2	1	11	1.6	++
19 F 45	2 yrs.	6	3	13	1.7	+
20 F 38	2 mos.	3	3	14	1.7	+
21 F 54	3 yrs.	1	0	7	0.8	+
22 F 41	3 yrs.	2	0	8	—	+
23 F 50	3½ yrs.	3	0	7	1.4	+
24 F 62	2½ yrs.	1	0	5	1.7	−
25† F 47	2 yrs.	5	0	9	—	−
	Post-thyroidectomy:‡					
26† F 59	11 mos.	3	1	12	0.8	++
27† F 38	9 mos.	3	1	10	1.7	++
28† F 67	6 mos.	2	0	8	1.2	++
29† F 46	3 mos.	4	1	8	1.6	+
30† F 61	3 wks.	2	0	5	1.7	−

* Normal ranges: T.t., 1–4 μ.; C.g., 0–2 μ.; Z.s., 3–11 μ.; γ-Glob., 0.7–1.6 Gm./100 ml.
† Histologically proved by biopsy up to Patient #25; by subtotal thyroidectomy in Patients #26–30.
‡ Cases 26–30 (up to one year after operation) are included in Table 2.

operation and with the length of time that had elapsed since thyroidectomy.

The results of precipitin tests performed on the sera of patients with other thyroid diseases are presented in Table 3. The findings were negative in 105 thyrotoxic patients whether active, postoperative, radioiodine-treated or in a state of prolonged remission after treatment with antithyroid drugs. In all of 103 patients with nontoxic nodular goiter the results were negative.

Precipitins were found in 6 cases of spontaneous myxedema without goiter in which there was no history of previous thyroid disease; but in 27 other cases of myxedema the results were negative.

TABLE 2. THYROGLOBULIN PRECIPITIN REACTIONS IN 25 CASES OF HASHIMOTO'S DISEASE TREATED BY THYROIDECTOMY OR X-RAY ABLATION OF THE THYROID

Treatment	Time elapsed since treatment	No. of cases	Precipitins
Hemithyroidectomy	Up to 1 year	1	++
	2 and 8 years	2	+, +
	10 years	1	−
Subtotal thyroidectomy	Up to 1 year	4	++, ++, +, −
	2–4 years	8	++, +, +, + −, −, −, −
	5–22 years	4	−, −, −, −
Total thyroidectomy	2–8 years	3	−, −, −
X-irradiation	3 and 5 years	2	−, −

Six patients had subacute thyroiditis of the giant-cell type. Precipitins were found in the sera of 4. In 2 of these, myxedema had developed many months after the acute illness (in 1 with persistent goiter, and in 1 without thyroid enlargement), but in 2 the precipitin was found three and five weeks, respectively, after the acute onset. We were able to follow 1 of these patients over a period of nine months. The titer of precipitin gradually rose from 1/2 to 1/16 five months after the onset of the illness, and

TABLE 3. THYROGLOBULIN PRECIPITIN REACTIONS IN OTHER THYROID DISEASES

	Number of cases		
	Positive	Negative	
Thyrotoxicosis			
Active or in remission after carbimazole therapy	0	68	
Post-thyroidectomy or post-I^{131}			105
Euthyroid	0	20	
Myxedema	0	17	
Nontoxic nodular goiter	0	103	
Myxedema without goiter	6	27	
Subacute thyroiditis (giant-cell type)	4	2	
Riedel's thyroiditis	0	1	
	10	238	

has now decreased to 1/4. This rise and fall in precipitin titer was confirmed by a sensitive quantitative method, using radioactive thyroglobulin. The patient has remained euthyroid.

DISCUSSION

The present results show that in the majority of cases the serum of patients with Hashimoto's disease gives a precipitin reaction when it is mixed with either crude or refined extracts of the human thyroid gland. This suggests an immunization against human thyroglobulin. An immunity of this nature might arise in response to a subtle structural alteration of the thyroglobulin present in the diseased gland, but certain facts are against this hypothesis. First, the antibody reacts very strongly with thyroglobulin obtained from normal glands. Secondly, Rose and Witebsky (22, 23) demonstrated that antibodies could be produced in normal rabbits by injection of extracts of the animal's own thyroid and that these antibodies reacted with the remaining lobe of the gland.

Structural alteration in a body constituent is not a prerequisite for the induction of auto-antibodies. The inability of animals to form antibodies against their own body constituents under normal conditions is considered to be the result of an immunologic tolerance acquired in early life, before the maturation of the faculty of immunologic response. Exposure to antigenic stimuli during this period causes a specific weakening or suppression of the immune reaction to these antigens in later life (24, 25). The concept of immunologic tolerance provides a rational basis for the phenomenon of auto-antibody formation, since animals may fail to acquire this tolerance for constituents which do not gain access to the sites of antibody formation during their critical developing period, and may therefore produce antibodies in response to any subsequent release of these constituents. The auto-antigenic properties of spermatozoa (26), lens protein (27). casein (28), and brain extracts (29) accord well with this theory. The failure to detect circulating thyroglobulin under normal conditions (30) suggests that human beings may not be immunologically tolerant to this protein and may react to its release from the thyroid by auto-antibody formation.

Auto-antibodies are known to cause histologic damage in organs against which they are directed. Thus aspermia has resulted from immunization with sperm extracts in guinea pigs (31) and encephalomyelitis has followed injection of brain extracts into monkeys. Rose and Witebsky found lymphoid infiltration and breakdown of follicles in the thyroid of rabbits immunized with homologous thyroid extracts (23).

The present demonstration of circulating antibodies against the patient's own thyroid in lymphadenoid goiter and the aforementioned destructive effects of auto-antibodies suggest a new approach to the etiology

of this disease. It may be supposed that limited extravasation of thyroglobulin (32) stimulates invasion by lymphoid tissue, with local antibody production. Under certain conditions sufficient antibody may be produced to damage adjacent follicles and release more thyroglobulin. This may in time give rise to a general immune response involving distant parts of the reticulo-endothelial system, with a considerable increase in antibody production, leading to further lymphoid hypertrophy and progressive damage to the thyroid gland. Classic Hashimoto's disease may represent a late stage in such a sequence of events, whereas lymphocytic thyroiditis, an analogous condition described in children and young people (33), is probably an earlier or milder form of the same pathologic process.

Localized lymphoid infiltration is a common finding in a variety of goiters (33, 35) and occurs in about 15 per cent of normal thyroid glands from elderly women (34, 36). The other histologic features of Hashimoto's disease, *e.g.*, Askanazy cell change (37), disintegration of follicular basement membrane (38), invasion of colloid by macrophages (34), and fibrosis, have all been observed to a lesser degree in other thyroid conditions and many authors consider these changes to be of the same nature as the more generalized lesion observed in classic Hashimoto goiters.

Thyrotoxicosis is frequently associated with localized lymphoid invasion and is known to precede the development of classic Hashimoto's disease in certain cases (39, 40, 41). In 2 of our cases of Hashimoto's disease with precipitins in the serum, there was a previous history of proved thyrotoxicosis. A correlation has also been noted between lymphoid infiltration of the thyroid and the development of postoperative myxedema. Greene (42) found that 38 per cent of patients with patchy lymphoid infiltration became myxedematous, compared with 0.3 per cent of patients showing no lymphoid changes. Histologic examination of the thyroid from a patient with postoperative myxedema in whom we found complement-fixing thyroid antibodies, showed scattered lymphoid follicles and epithelial damage. If this represented a localized immune response, the thyroglobulin released at operation (30) would be expected to act as a boosting dose and to stimulate increased antibody production, with consequent destruction of the residual thyroid tissue.

Similarly, after therapeutic doses of I^{131}, thyroidal histologic patterns indistinguishable from those in Hashimoto's disease have been observed (43) and thyroglobulin is known to be released, suggesting that autoimmunization may play a role in the onset of hypothyroidism, particularly when it occurs after treatment with relatively small doses of the isotope.

The demonstration of precipitins in 2 early cases of subacute giant-cell thyroiditis (1 proved by biopsy) is of particular interest, since in this disease thyroglobulin is thought to escape into the circulation (44) as a result

of follicular damage due to a virus infection, and focal lymphoid changes are sometimes seen in the thyroid. Though in most patients the disease subsides without complications, the development of auto-immunity may account for puzzling cases (4) which present a clinical picture of subacute thyroiditis, yet later prove to be Hashimoto's disease.

Our results further suggest that spontaneous myxedema without goiter may be the end-result of thyroid auto-immunity. Shrunken thyroid glands with extensive lymphadenoid changes were described by Simmonds (45) in elderly women with hypothyroidism. The size eventually attained by a goiter resulting from the thyroid auto-immunization process, must depend on individual variation in the pituitary output of thyroid-stimulating-hormone (TSH) and on the capacity of the thyroid gland to hypertrophy in response to thyrotropic stimulation, as well as on the intensity of the immune response.

Overstimulation of the thyroid by TSH has been stressed as an important factor in the pathogenesis of lymphadenoid goiter (2, 35). Inactivation of thyroglobulin by reaction with the auto-antibody and the resultant depletion of circulating thyroid hormones would be expected to stimulate production of TSH, with consequent hyperplasia of the thyroid and enhancement of the antibody response. This would explain the strong precipitin reactions obtained in untreated patients with large goiters. Administration of desiccated thyroid to patients with Hashimoto's disease produces a striking reduction in the size of the goiter by inhibiting TSH and thereby reversing this chain of events. The beneficial effect of cortisone may perhaps depend on its inhibition of antibody production.

After thyroidectomy for Hashimoto goiter, the antibody level gradually decreases, and precipitin reactions are negative in patients operated on several years previously. This reflects the decrease in antigenic stimulus, which is further diminished by thyroid therapy.

It appears that, although classic Hashimoto's disease is comparatively rare, it represents but an extreme degree of a phenomenon which seems to be more widespread. The efficacy of thyroid therapy and the diagnostic value of the thyroglobulin precipitin reaction in conjunction with serum protein-flocculation tests should obviate the need for operation in patients with Hashimoto's disease.

SUMMARY

The serum of patients with Hashimoto's disease contains auto-antibodies against thyroglobulin, which have been demonstrated by diffusion-precipitation reactions in agar gels. The antibody is organ-specific and does not cross-react with extracts of the thyroid gland from any of six mammalian species.

The antibody level was highest in untreated patients; weaker or negative precipitin reactions were obtained in a group of 25 Hashimoto patients after thyroidectomy.

Thyroglobulin precipitins were also found in the sera of 6 patients with spontaneous nongoitrous myxedema and 4 patients with subacute (giant-cell) thyroiditis. No precipitins were found in the sera of 238 patients with various thyroid disorders.

It is suggested that destruction of the thyroid in Hashimoto's disease results from progressive interaction of the auto-antibody with thyroglobulin in the gland, and that lesser degrees of lymphoid infiltration found in other thyroid diseases may represent a localized immune response.

Addendum

We have now studied a total of 144 Hashimoto patients who were not operated on; in 109 of these, results of the precipitin test were positive. An additional 3 cases of subacute thyroiditis have been studied; in 1 (proved by biopsy) the results of the precipitin test were positive up to 1/32 dilution of serum, but in the other 2 the results were negative. Thyroglobulin precipitins have now been found in a case of active untreated thyrotoxicosis.

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FACTORS AFFECTING THE BINDING CAPACITY OF HUMAN ERYTHROCYTES FOR I^{131}-LABELED *l*-THYROXINE AND *l*-TRIIODOTHYRONINE*†

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THE purpose of this paper is to present further studies on the binding or uptake of I^{131}-labeled thyroid hormones by human erythrocytes. Previous studies from our laboratory (1, 2) have shown that the presence of human plasma markedly inhibits the uptake of *l*-thyroxine and to a lesser extent *l*-triiodothyronine. This inhibition was considered to be due to the binding of *l*-thyroxine by the "thyroxine-binding protein" of plasma. The "thyroxine-binding protein" has not been isolated, but it has been shown by several investigators (3–6) that at physiologic concentrations thyroxine is principally associated with a protein which at pH 8.6 displays an electrophoretic mobility intermediate between those of $alpha_1$ and $alpha_2$ globulins. Data will be presented to show that human serum albumin inhibits the uptake by the red cell of radioactive *l*-thyroxine and *l*-triiodothyronine, but to a lesser degree than by an equal amount of human plasma, and that human gamma globulin causes no significant decrease in the uptake of radioactive *l*-thyroxine by the red cells.

It will also be shown that: 1) the addition of stable *l*-thyroxine to plasma decreases its binding properties for radioactive *l*-thyroxine while apparently not affecting the binding sites on the red cell, 2) the duration of contact of the hormone with the red cell is a factor influencing the ultimate uptake of labeled thyroid hormones, and 3) the vital dyes, Evans Blue and Trypan Blue differ in their effects on the binding of radioactive *l*-thyroxine by red cells and by plasma.

MATERIALS AND METHODS

Blood from healthy volunteers was drawn in heparinized syringes, placed in a 50-ml. centrifuge tube, and centrifuged for fifteen minutes at 1500 rpm. The plasma was drawn off, the cells resuspended in buffered[1] saline solution, recentrifuged, and the saline solution siphoned off and discarded. The latter procedure was repeated twice. Plasma obtained from a healthy volunteer, was mixed with buffered saline (pH 7.3) to yield a 1 in 10 dilution. The concentration of total protein in this plasma was 7.6 Gm. per 100

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[1] Sorenson Buffer (Difco—0512–33).

ml., and that of albumin was 4.1 Gm. per 100 ml. Human gamma globulin was also diluted (1 in 10) with buffered saline, and human serum albumin[2] (purified) was suspended in buffered saline to yield solutions containing 4.1 and 7.6 Gm. per 100 ml., respectively. I^{131}-labeled *l*-thyroxine and *l*-triiodothyronine[3] solutions were also diluted with buffered saline, so that 2 ml. of the diluted solutions registered approximately 2.5×10^4 counts per minute.

Two-ml. samples of the radioactive labeled hormones were placed in 15-ml. test tubes and counted in a well counter. To each was added 1.0 ml. of the diluted plasma solution, followed by gentle mixing. After addition of 2.0 ml. of the washed red cells to each tube, the solutions were gently mixed. The mixture was incubated at 37° C. for thirty minutes and then centrifuged to separate the cells from the plasma. The cells were washed three times with buffered saline by resuspension and recentrifugation, and then counted in a well counter. This count divided by the count for the original 2 ml. of radioactivity represented the percentage of labeled hormone taken up by the red cell. This procedure was repeated using 0.5 ml. and 0.1 ml. of the diluted plasma and 1.0 ml. each of the gamma globulin and albumin solutions.

The same procedure was repeated, except that 0.5, 1.0 and 2.0 ml., respectively, of Evans Blue dye (0.5 per cent aqueous solution) was mixed with 1.0 ml. of the plasma (1/10 dilution) and allowed to stand for fifteen minutes before the plasma was added to the radioactive labeled *l*-thyroxine. A 0.5 per cent solution of Trypan Blue was also used in exactly the same manner.

The effect of time on the uptake was determined by adding 2 ml. of red cells to 2 ml. of previously counted radioactive *l*-thyroxine or *l*-triiodothyronine. The whole was gently mixed and then incubated at five, ten, twenty and thirty-minute intervals. The cells were then washed as previously described and the results reported as the percentage uptake by the cell.

Solutions of stable *l*-thyroxine in .02 *N* NaOH were prepared so that 1 ml. was equivalent to 1.3, 6.4 and 32 micrograms, respectively. One ml. of each of the foregoing solutions was added to 2.0 ml. of the previously counted solution of radioactive *l*-thyroxine, followed by the addition of 2 ml. of red cells. The solution was gently mixed, incubated at 37° C. for thirty minutes, and then centrifuged to separate the cells from the plasma. The cells were washed three times with buffered saline and counted in a well counter. The results were presented in the usual fashion.

The same procedure was repeated, except that 0.5, 1.0 and 2.0 ml. respectively of Evans Blue dye (0.5 per cent aqueous solution) was added to the previously counted radioactive solution of *l*-thyroxine before the red cells were added. In a similar procedure, 0.5 per cent solution of Trypan Blue was used in place of the Evans Blue.

Solutions of stable *l*-thyroxine were prepared in .02 *N* NaOH so that each 0.1 ml. was equivalent to .08, .16 and .64 microgram, respectively. One ml. of human plasma diluted (1 in 10) with buffered saline was added to 1.0 ml. of the foregoing solutions, and then this mixture was added to 2 ml. of previously counted radioactive *l*-thyroxine, followed by the addition of 2 ml. of red cells. The whole was gently mixed, incubated for thirty minutes at 37° C., and centrifuged to separate the cells from the plasma. The cells were then washed three times with buffered saline and counted in a well-counter.

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[2] Obtained through the courtesy of Dr. A. Chanutin, Department of Biochemistry of University of Virginia, Charlottesville, Virginia.

[3] Obtained from Abbott Laboratories, Oak Ridge, Tennessee.

RESULTS

The uptake of radioactive *l*-thyroxine[4] by the red cells averaged 74.8 per cent and that of *l*-triiodothyronine[5] averaged 82 per cent (Table 1). The presence of plasma reduced the erythrocytic uptake of radioactive *l*-thyroxine as follows: 0.1 ml. reduced it to 48.5 per cent, 0.5 ml. to 20 per cent, and 1.0 ml. to 10.6 per cent. The latter solution reduced the uptake of radioactive *l*-triiodothyronine to 56.5 per cent.

The erythrocytic uptake of radioactive *l*-thyroxine was reduced to 29 per cent by 1 ml. of albumin solution, containing 7.6 mg., and to 36.0 per

TABLE 1. UPTAKE BY RED CELLS IN THE PRESENCE OF HUMAN PLASMA OR ALBUMIN

Mat'l added before incubation→	Radioactive *l*-thyroxine						Radioactive *l*-triiodothyronine		
	Control	Human plasma diluted 1 in 10			1 ml. human serum albumin		Control	1 ml. human plasma diluted 1 in 10	1 ml. human albumin (1 ml.≃ 4.1 mg.)
		0.1 ml.	0.5 ml.	1.0 ml.	1 ml.≃ 4.1 mg.	1 ml.≃ 7.6 mg.			
Per cent uptake by R.B.C.									
Avge.	74.8	48.5	20.0	10.6	36.8	29.0	82.0	56.5	68.3
Range	70–80	42–52	15–24	8–15	31–42	26–34	80–88	53–60	65–72
No. of determinations	151	20	22	86	28	24	37	28	24

cent by 1 ml. of albumin solution containing 4.1 mg. The latter solution reduced the uptake of *l*-triiodothyronine to 68.3 per cent. One ml. of gamma-globulin solution caused no significant decrease in the uptake by the red cells of either radioactive *l*-thyroxine or *l*-triiodothyronine.

The addition of stable *l*-thyroxine to plasma before adding it to the erythrocyte-radioactive thyroxine mixture caused a significant increase in the uptake of radioactive *l*-thyroxine by the red cells. However, the addition of 1 ml. of a stable thyroxine solution containing either 1.3, 6.4 or 32 micrograms produced no significant change in the erythrocytic uptake of radioactive *l*-thyroxine in the absence of plasma (Fig. 1).

The effect of time of incubation on the erythrocytic uptake of radioactive *l*-thyroxine in the absence of plasma and on the uptake of radioactive *l*-triiodothyronone in the absence of plasma are shown in Figure 2.

The addition of 0.5 ml. of Evans Blue reduced the erythrocytic uptake of *l*-thyroxine to 55.6 per cent, 1.0 ml. reduced it to 45.0 per cent, and 2.0

[4] In 1 ml. there was approximately 1.0×10^{-4} μg. of *l*-thyroxine.

[5] In 1 ml. there was approximately 0.5×10^{-3} μg. of *l*-triiodothyronine.

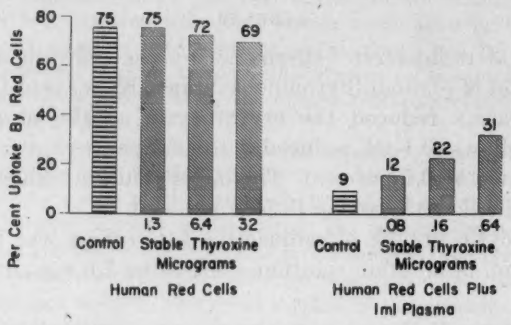

FIG. 1. The effect of stable *l*-thyroxine on the uptake of radioactive *l*-thyroxine by human red blood cells and by red blood cells plus plasma.

ml. reduced it to 37.1 per cent (Fig. 3). Trypan Blue produced no significant change in the uptake of *l*-thyroxine by the red cells.

The addition of as much as 2.0 ml. of Evans Blue to the plasma did not increase the erythrocytic uptake of the *l*-thyroxine. However, the addition of 0.5 ml. of Trypan Blue to the plasma increased the uptake of *l*-thyroxine to 24.0 per cent, 1.0 ml. increased it to 31.2 per cent, and 2.0 ml. to 37.4 per cent (Fig. 4).

DISCUSSION

It has been shown by several investigators that under physiologic conditions thyroxine is found chiefly in association with a plasma protein having electrophoretic mobility (at pH 8.6) between that of alpha$_1$ and alpha$_2$ globulins. This thyroxine-binding protein (T.B.P.) or thyroxine-binding globulin (T.B.G.) has not been isolated in pure form. Of the various plasma components which have been tested, Cohn's fraction IV-6 appears to have the greatest binding capacity (7). Albright, Larson and

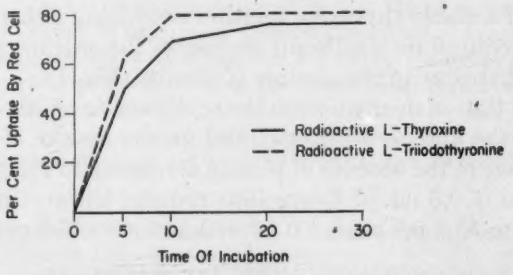

FIG. 2. The effect of duration of incubation on the uptake of radioactive *l*-thyroxine and *l*-triiodothyronine by red blood cells.

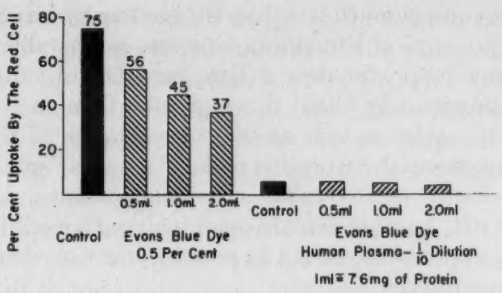

FIG. 3. The effect of Evans Blue on the uptake of radioactive *l*-thyroxine by human red blood cells and by red blood cells plus plasma.

Deiss demonstrated that when exogenous *l*-thyroxine is added to plasma *in vitro* in quantities exceeding 0.1 microgram per liter, the thyroxine can be found in all protein fractions, with albumin as the main secondary carrier (8).

Previous studies (1, 9) had shown that, *in vitro*, the red cell would bind both *l*-thyroxine and *l*-triiodothyronine. The studies reported here show that a time factor is involved, in that the maximum erythrocytic uptake of *l*-triiodothyronine occurred between five and ten minutes, and that for *l*-thyroxine between ten and twenty minutes; in addition, the inhibition of erythrocytic uptake of thyroid hormones by the presence of plasma was again demonstrated (2). This inhibition appears to be semi-quantitative, as the amount of thyroxine bound by the plasma increases as the amount of plasma added to the incubation mixture is increased. It should be emphasized that the degree of binding by plasma is estimated by a decrease in the erythrocytic uptake of the radioactive label as compared to the control without plasma. Likewise, a decrease in the binding capacity of plasma is estimated by an increase in the erythrocytic uptake of the radioactive label as compared to a control with the same amount of plasma.

This study demonstrates that the binding of *l*-thyroxine and *l*-triiodo-

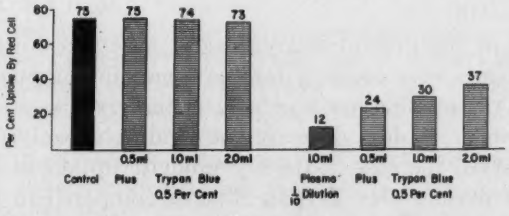

FIG. 4. The effect of Trypan Blue on the uptake of radioactive *l*-thyroxine by human red blood cells and by red blood cells plus plasma.

thyronine by albumin in much less than the binding by an equal amount of plasma. In the presence of albumin in amounts comparable to that found in normal plasma (approximately 4 Gm. per 100 ml.) the erythrocytic uptake was approximately three times greater than in the presence of plasma. One explanation is that as the concentration of thyroxine is increased in the plasma with saturation of T.B.G. and a "spilling over" onto albumin, this moiety of thyroxine or triiodothyronine would be more available to the cell. Another explanation involves an equilibrium between free thyroxine and thyroxine bound to protein. Our data do not allow us to make this differentiation.

It has been suggested that the distribution of thyroid hormones between serum and cells is dependent upon the physico-chemical binding properties of the serum versus those of the cell (2, 11). Expressed in a different manner, the availability of thyroid hormones to the cell depends upon the relative binding capacity of the serum and perhaps the specific binding proteins for thyroxine in the cell. The results of the present studies indicate that as the thyroxine content of plasma is increased there is saturation of the binding points of the T.B.G., which in turn would make more thyroxine available to the cell because of the weaker binding capacity of the secondary carrier, albumin. This might account, in part, for the faster disappearance of labeled *l*-thyroxine from the serum of patients with hyperthyroidism, as reported by Sterling and Chodos (10), and for the increased erythrocytic uptake of labeled *l*-triiodothyronine from the plasma of patients with Graves' disease as reported by Hamolsky (9). There appears to be no binding of thyroxine by gamma globulin, as the erythrocytic uptake was not changed in the presence of this component of plasma.

Larson and Albright (13) and Robbins and Rall (14) investigated the specificity of the binding of thyroxine by thyroxine-binding protein of serum, using various analogues of thyroxine. The technique was devised to show that the various analogues of thyroxine would displace or compete with thyroxine for the binding sites on T.B.P. The three groups—amino, carboxyl and phenol—appear to be essential for the binding of thyroxine, since the absence of any one of them reduces markedly the binding affinity of the analogue (13).

The findings of the present study suggest that substances other than thyroxine analogues may compete for the same binding points on plasma as thyroxine. Trypan Blue has no effect on the erythrocytic uptake of *l*-thyroxine. However, it does decrease the binding capacity of plasma, as shown by a marked increase in the erythrocytic uptake of *l*-thyroxine in the presence of plasma plus Trypan Blue as compared to plasma alone. It is of interest that Taurog and associates (15) found that thyroxine became localized in the same area of the brain as did Trypan Blue. Evans

Blue appeared to compete with *l*-thyroxine for binding points on the red cell, as shown by the decreased uptake of the radioactive *l*-thyroxine. However, it did not appear to compete with *l*-thyroxine for binding points on plasma, as there was no change when compared with the control. That actual competition takes place between Trypan Blue and *l*-thyroxine for the same binding points is currently being investigated using a radio-electrophoretic technique.

It is to be emphasized that these studies are not concerned with thyroxine metabolism in the red cell. The red cell is used as model of any cell which will bind thyroid hormones. This assumption seems justified in view of the fact that similar results have been reported by Freinkel, Ingbar and Dowling (11) using kidney, liver and heart and by Klemperer using liver mitochrondria (12).

SUMMARY

1. The uptake by the red cell of I^{131}-labeled thyroid hormones was inhibited by serum albumin but to a lesser extent than by an equal amount of plasma. Serum gamma globulin had no effect on the erythrocytic uptake of I^{131}-labeled *l*-thyroxine.

2. Trypan Blue appeared to compete with *l*-thyroxine for binding points on the "thyroxine-binding protein," whereas Evans Blue had no effect.

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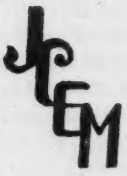

A CHROMATOGRAPHIC STUDY OF THYROIDAL IODINE METABOLISM IN NONTOXIC NODULAR GOITER*

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NONTOXIC nodular goiter is thought to result from various physiologic and pathologic stimuli; the mechanism of the changes is unknown. Thyroid adenomas have been given many histologic descriptions but these cannot yet be related either to the etiology of the nodules or to their function. The large single nodule, an adenoma, may render the rest of the gland inactive, and itself be solely the source of the patient's circulating hormone (1). Nodules which have a high uptake of I^{131} have been described as "hot," but nothing is known of their biochemical function until thyrotoxicosis develops—when it is presumed that they are secreting an excess of thyroxine.

Some years ago, Cavett, Rice and McClendon (2) showed that thyroglobulin in patients with colloid goiter usually contained less total iodine and less thyroxine iodine than in euthyroid subjects. The total iodine, as well as the thyroxine iodine, was only 10–20 per cent of that found in thyroglobulin obtained at autopsy from subjects not known to have any thyroid disorders.

Later Leblond and co-workers (3) studied the uptake of I^{131} by human goitrous thyroids and the distribution of I^{131} between thyroxine, diiodotyrosine and iodide, using butanol fractionation. They found that in 4 cases of nontoxic nodular goiter and in 1 mildly toxic case, the iodine content of the adenomatous tissue was lower than that of the surrounding tissue; the thyroxine content was also lower.

In the present study, the distribution of iodine between the iodinated amino-acids of the thyroid gland has been investigated with the aid of I^{131} and chromatographic analysis. Preliminary experiments using autoradiography of the chromatograms revealed unexpectedly large amounts of monoiodotyrosine (MIT) compared with diiodotyrosine (DIT), together with little or no thyroxine. It was therefore thought of interest to investigate quantitatively the amounts of I^{131} incorporated into these amino-

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acids at different time-intervals after administration of I^{131}, and to compare the findings with those obtained in subjects without thyroid abnormalities.

MATERIALS AND METHODS

With 2 exceptions (Patient 9, courtesy of Professor P. S. Pilcher, University College Hospital, and Patient 10, courtesy of Mr. Selwyn Taylor, Hammersmith Hospital), the 15 goitrous patients studied were from the Derbyshire Royal Infirmary. All the patients were females. From one to four days before undergoing thyroid surgery, each was given about 200 μc. of I^{131}. Thyroidal I^{131} uptakes were determined either following a preliminary small tracer dose or following the 200-μc. dose.

Three subjects with no evidence of thyroid disorder were also studied. Patient 16 from the Derbyshire Royal Infirmary received 52 μc. of I^{131} before being explored for a parathyroid adenoma. Patients 17 and 18 (courtesy of Mr. Douglas Ranger, Middlesex Hospital) received 200 μc. and 520 μc. of I^{131}, respectively, before undergoing block dissections of the neck for carcinoma not involving the thyroid gland.

After surgery, the fresh samples of gland were treated as soon as possible with alkali or with trypsin. Alkaline hydrolysis consisted of a modification of the method of Harington and Randall (4) whereby about 2 Gm. of fresh thyroid was boiled under reflux for sixteen to eighteen hours with 2*N* NaOH (10 ml.). Tryptic hydrolysis was carried out by the method of Gross and Pitt-Rivers (5). "Thyroxine iodine" values were obtained according to the procedure of Harington and Randall, by acidifying an aliquot of the hydrolysate to pH 2–3 and allowing the thyroxine to precipitate for sixteen to twenty hours. The rest of the hydrolysate was acidified to pH 2–3, extracted once with chloroform to remove fat, and then three times with n-butanol. The concentrated butanol extracts were analyzed by ascending chromatography in n-butanol saturated with 2*N* acetic acid or in n-butanol-dioxan-ammonia (2*N*), 4:1:5. The chromatograms were then passed through an automatic strip counter attached to a recorder. Measurements of the areas covered by the different peaks yielded an estimate of the amounts of I^{131} contained in the various iodinated amino-acids and iodide.

Later experiments were performed in order to determine whether there were any differences in the distribution of I^{131} in the iodinated compounds from the nodules compared with those from the paranodular tissue. These tissues were therefore separated after operation and analyzed independently.

In 4 patients, samples of serum taken immediately before operation were extracted with n-butanol after acidification to pH 2–3, and analyzed by chromatography.

RESULTS

Thyroidal I^{131} uptakes. In all but 1 patient (No. 12) the uptakes were in the normal range. The low uptake in Case 12 was probably due to the fact that the patient had been receiving iodine medication up to two months before operation.

Thyroidal thyroxine I^{131} concentration. Autoradiographs of chromatograms of the early thyroidal hydrolysates (Patients 1, 2 and 3, Table 1) failed to reveal detectable amounts of thyroxine, and later measurements obtained from the strip counter showed that amounts of labeled thyroxine, either in the so-called "normal" thyroid tissue or in the nodules, were in

TABLE 1. DISTRIBUTION OF I^{131} IN PARANODULAR TISSUE (T) AND IN NODULES (N) IN NONTOXIC GOITROUS PATIENTS

Case No.	Dose of I^{131} (μc.)	Days before operation	I^{131} (% dose per Gm. tissue)	Thyroxine I^{131} (%)	$\frac{I^{131}\ MIT}{DIT}$
1	192	1	—	<5*	3.0
2	252	1	—	<5*	0.9
3	190	1	—	<5*	1.2
4	207	1	—	2	1.3
5	185	1	T 1.39 N 0.06	10 3	0.7 3.0
6	218	2	T 1.60 N 0.16	8 5	0.7 6.0
7	232	2	T 0.08 N 0.14	0 6	0.7 0.7
8	217	2	N 1 0.4 2 0.6	10 5	— —
9	280	2	0.12	7	1.0
10	100	2	T 0.31 N 0.17	21 0	0.65 1.2
11	191	3	0.69	9	0.6
12	218	3	T 0.024 N 0.006	12 1	— —
13	202	3	T 0.46 N 0.43	8 6	2.3 2.3
14	188	4	T 0.18 N 1.69	11 23	0.8 0.4
15	218	4.2	T 0.75 N 0.27	7 5	— —

* Values estimated from autoradiographs.

TABLE 2. DISTRIBUTION OF I^{131} IN THYROID TISSUE FROM NORMAL SUBJECTS

Case No.	Dose of I^{131} (μc.)	Days before operation	I^{131} (% dose per Gm. tissue)	Thyroxine I^{131} (%)	$\frac{I^{131}\ MIT}{DIT}$
16	52	0.75	—	10	0.75
17	200	1	2.8	11	0.6
18	520	2	1.6	24	0.5

general considerably lower than those found in the normal subjects (Table 2). In only 2 patients (Nos. 5 and 10) was there a normal content of labeled thyroxine in the histologically "normal" tissue. A single high value for the thyroxine content of a nodule was found in Patient 14; this patient also had symptoms of mild hyperthyroidism. Figure 1 shows the thyroxine-I^{131} per cent of total I^{131} plotted against the time that the I^{131} was in the gland; it can be seen that the thyroxine content of the "normal" tissue of the goiters reached a plateau soon after two days and did not change up to four days. No definite conclusion about the distribution of stable iodine can be drawn from these experiments, but it seems unlikely that any great

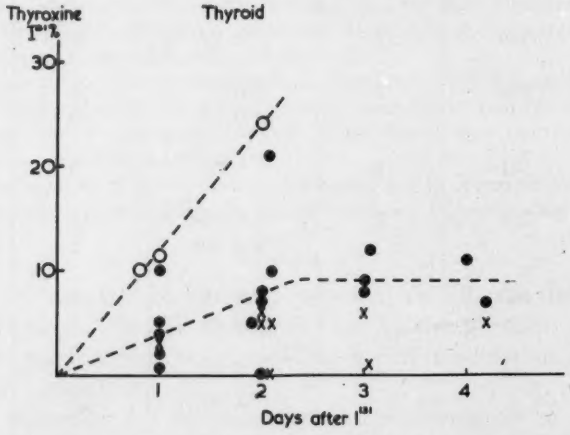

FIG. 1. Thyroidal thyroxine I^{131}% in normal subjects and in patients with nodular goiter at different time-intervals after administration of I^{131}. (Open circles represent thyroid tissue from normal subjects. Solid circles represent paranodular tissue from goiters. Crosses represent nodular tissue.)

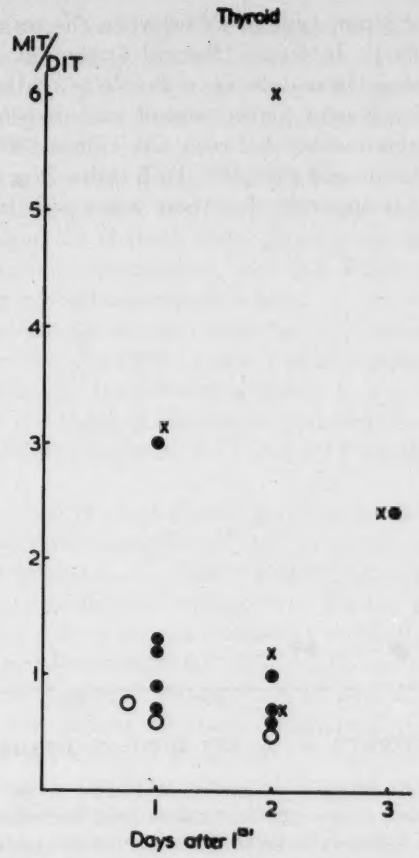

FIG. 2. MIT/DIT ratios in normal subjects and in patients with nodular goiter at different time-intervals after administration of I^{131}. (Open circles represent thyroid tissue from normal subjects. Solid circles represent paranodular tissue from goiters. Crosses represent nodular tissue.)

rise in thyroxine content would be demonstrable if I^{131} remained for a longer time in the gland. It is also obvious from Figure 1 that most of the nodules contained less thyroxine than did the paranodular tissue.

Distribution of I^{131} between MIT and DIT. A comparison of the MIT: DIT ratios in normal thyroid glands and in nodular goiters is shown in Figure 2. It is apparent that in all the goiters this ratio was higher than that found for the thyroids of the 3 normal subjects, and even for the paranodular ("normal") tissue of the goiter patients, although not es-

pecially marked for either type of tissue when the total iodine (I^{131}) content was low (Table 1). In 1 case (Patient 6) this ratio reached the high level of 6; nevertheless the nodule was still able to synthesize thyroxine.

Relationship between total iodine content and distribution of iodinated amino-acids. The relationship between the values for labeled thyroxine I^{131}% per gram of tissue and the MIT:DIT ratios (Fig. 3) is perhaps even more significant. It is apparent that there was a wide range of MIT:DIT

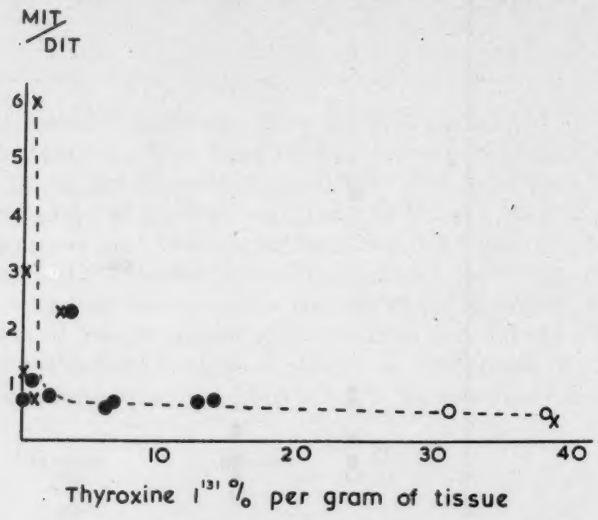

FIG. 3. Relationship between I^{131}-labeled thyroxine % per gram of tissue and MIT/DIT ratios. (Open circles represent values from normal subjects. Solid circles represent values from paranodular tissue. Crosses represent values from nodules.)

values in the nodules—all associated with a low thyroxine content. In paranodular tissue, normal or elevated MIT:DIT ratios were also associated with low thyroxine values. In the normal glands, low MIT:DIT ratios were associated with high thyroxine contents. It is of interest that in the "hot" nodule from Patient 14 the ratio was at the upper end of the normal range.

Analyses of serum. In the 4 sera examined by chromatographic analysis (Patients 1, 2, 3 and 4) thyroxine was found. Iodide was also present in the serum of Patient 2, but in none was there any evidence of DIT or MIT.

DISCUSSION

It is now generally accepted that the biosynthesis of thyroid hormone takes place according to the following reactions: (i) Iodide is concentrated

in the gland from the blood. (ii) The trapped iodide after oxidation is bound first to tyrosine in thyroglobulin—first as MIT and then as DIT. (iii) Two molecules of DIT are coupled to yield thyroxine. (iv) Triiodothyronine is probably formed by a similar mechanism, from one molecule each of DIT and MIT.

Thereafter, thyroxine and triiodothyronine are liberated from thyroglobulin by thyroidal protease, and they pass into the circulation. MIT and DIT do not leave the thyroid under physiologic conditions: they are deiodinated by thyroidal deiodinase, and the iodide so formed is presumably re-utilized in the biosynthetic cycle.

Three biosynthetic defects have now been demonstrated in familial goitrous hypothyroidism: the first, a defect in incorporation of iodide into organic combination (6); the second, a defect in conversion of DIT to thyroxine (7); and the third, a combination of defects—a failure to synthesize thyroxine and to deiodinate MIT and DIT which are liberated into the circulation (8).

It has been suggested (9) that simple goiter is caused by failure of the gland to obtain adequate supplies of iodine, resulting from a dietary deficiency or from ingestion of dietary goitrogens. Our results indicate that iodine deficiency alone does not account for the growth of nodular goiters. It is true that these goiters manifest a reduced capacity to utilize concentrated iodine; this is especially marked in the nodules. However, in Patient 6, whose "normal" thyroid tissue had an I^{131} content and MIT:DIT ratio very similar to those in thyroid tissue from normal Subject 18, the concentration of thyroxine I^{131} was less than half that in the control. In this case, the biosynthetic deficiency seemed to have been restricted to the stage of thyroxine formation.

Several of the patients in this study were able to synthesize labeled thyroxine in spite of a very low concentration of thyroidal I^{131}. This was particularly marked in Patient 10, in whose thyroid the I^{131} level was only one-fifth of that in the corresponding normal subject (Subject 18); nevertheless the conversion of DIT to thyroxine was almost as efficient as in the control. Similarly Patients 7, 8, 9 and 12, with thyroidal I^{131} concentrations of only 0.02–0.6 per cent of the dose per gram of tissue (one-twentieth to one-third of the normal concentration) also converted DIT to thyroxine to some extent. Stanley (10) reported a case of thyroid carcinoma in which the MIT:DIT ratio in the malignant tissue was as high as 5 two days after administration of I^{131}; no thyroxine or triiodothyronine was found. High MIT:DIT ratios may sometimes result from the low concentrations of iodine in these glands, although this is not the only possible explanation. It may be that the enzyme which activates diiodotyrosine synthesis is relatively ineffective in these cases. Evidence in favor of this

hypothesis is found in the analysis of the goiter from Patient 9; here a very low concentration of iodide, less than one-tenth of normal, was not accompanied by an unusually high MIT:DIT ratio, so one must suppose that the diiodotyrosine-synthesizing enzyme was able to compensate for the deficiency to some extent.

In spite of these biochemical faults, it should be emphasized that all the patients except Number 14 were clinically euthyroid and that thyroxine was found in the sera of 4 of them. The small amounts of thyroxine

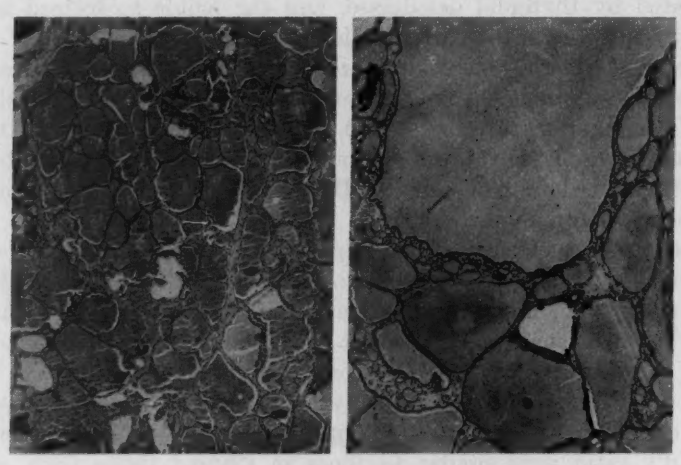

FIGURE 4

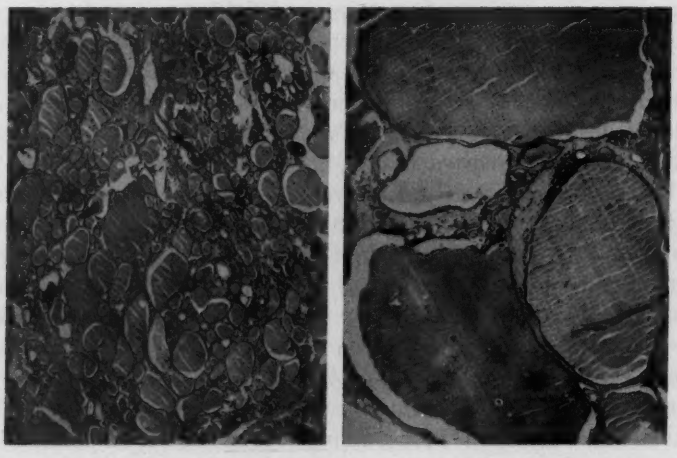

FIGURE 5

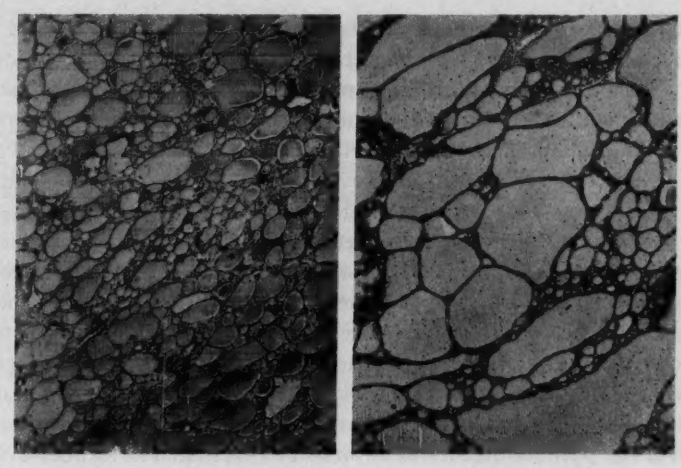

FIGURE 6

Histologic appearance of paranodular tissue (left) and of nodule (right)
FIG. 4. From Patient 6.
FIG. 5. From Patient 13.
FIG. 6. From Patient 7.

synthesized by the goiters were probably only adequate to maintain the patients in this state, and did not allow for storage in thyroglobulin. A more marked example of this failure to synthesize thyroxine is found in the family of goitrous cretins described by Hubble (11); I^{131}-labeled thyroxine was found in the serum and thyroid of 1 of these patients, but the amounts were not enough to prevent a state of extreme hypothyroidism.

In the present series of cases, examination of sections from some of the glands did not show any correlation between histologic appearance and biochemical function. Figure 4 is a photomicrograph of paranodular and nodular tissue from Patient 6; in this case the nodule which showed marked hyperinvolution was the more biochemically feeble. In Figure 5 is shown the histologic picture of the thyroid in Patient 7; the paranodular tissue contained no thyroxine, whereas the nodule (which also was deficient in iodine) had a labeled-thyroxine content which amounted to 6 per cent of the total I^{131}. In Patient 13 (Fig. 6) sections of paranodular and nodular tissue showed striking differences in appearance; nevertheless the biochemical performances of the two types of tissue were almost indistinguishable.

In conclusion, this work suggests that nodular goiters result from inability of the thyroid tissue to synthesize the amounts of thyroxine which

are normally required for body needs and for storage in the gland. This biochemical insufficiency, which precedes the formation of the nodules, probably continues during their growth. The stimuli which initiate the biochemical insufficiency are still unknown, but the immediate cause seems to be a falling off in the activity of the enzymes involved in the various stages of hormone synthesis. However, other factors may account for nodule formation. This is suggested by the results of analysis of the thyroid tissue from Patient 5; here the concentration of I^{131} in paranodular tissue was normal, and the thyroxine content and MIT:DIT ratio were only slightly elevated. In this instance, therefore, depression of hormone production did not seem to have been the precipitating cause of nodule formation, and some other explanation must be found. It is conceivable that this thyroid gland at one time did not produce the necessary amount of hormone for health, and that after nodules had begun to be formed, the function of the paranodular tissue returned to normal. The slender evidence for such a hypothesis will need experimental support; this might be forthcoming if more cases of this kind were found.

SUMMARY

1. Thyroidal iodine metabolism in nontoxic nodular goiter has been studied with the aid of chromatographic analysis after the administration of I^{131} to patients undergoing thyroid surgery.

2. A low concentration of I^{131} was generally found, both in the nodules and in the paranodular tissue.

3. The thyroxine content of both types of thyroid tissue was usually diminished.

4. The ratio of monoidotyrosine to diiodotyrosine was elevated, sometimes to very high values.

Acknowledgments

We are grateful to Mr. Douglas Ranger for supplying us with 2 samples of normal thyroid tissue; to Mr. J. R. Ratcliffe, Mr. A. J. Wilson and Mr. J. P. Collins, who removed the goiters; to Dr. G. R. Obsorn for the histologic sections; and to Mr. M. R. Young for the photomicrographs.

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THE METABOLISM OF 3:3′-DIIODOTHYRONINE IN MAN*

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MONOIODOTYROSINE, diiodotyrosine, 3:5:3′-triiodothyronine and thyroxine are native substances in the thyroid gland. Normally, the latter two are secreted into the blood as the definitive thyroid hormones, but mono- and diiodotyrosine escape into the blood only under unusual circumstances (1). The metabolic fate of each of these substances has been observed after intravenous administration in man. Mono- and diiodotyrosine are rapidly degraded (2, 3), whereas thyroxine and triiodothyronine disappear more slowly—thyroxine with a half-time of approximately seven days (4) and triiodothyronine with a half-time of two to three days (5).

Recently, evidence has been offered for the existence of 3:3′-diiodothyronine (3:3′-Th) in the thyroglobulin of the rat (6, 7), where it is said to account for as much as 25 per cent of the iodine previously attributed to thyroxine. Similarly it is said to account for as much as 25 per cent of the plasma iodine which previous methods of chromatographic separation had failed to resolve (8). The present studies describe the fate and excretion of 3:3′-Th after intravenous administration to subjects with and without thyroid disease. It will be shown that this substance is rapidly degraded. Its iodine appears in the urine almost entirely as free iodide.

MATERIALS AND METHODS

The 10 subjects were selected from the wards and the clinic for ambulatory patients. Several were studied on the metabolic ward in order to insure accuracy of urine collections. Three had typical Graves' diseases, as proved by the usual clinical and laboratory evidence. One (No. 4) was a patient with goiter and hypothyroidism who had normal stature and intelligence; several siblings were similarly affected. The remaining subjects had no evidence of thyroid disease.

Both *dl*-3-monoiodothyronine and *dl*-3:3′-diiodothyronine (3:3′-Th) were prepared and generously supplied by Dr. Chalmers Gemmill (9). I^{131}-labeled 3:3′-Th was prepared by iodination of 3-monoiodothyronine. A mixture of 200 μg. of KI and 5 mc. of I^{131} was acidified with 0.1 ml. of *N* HCl and oxidized with 0.4 ml. of 0.8 per cent H_2O_2. The iodine was collected in 1 ml. of ether. The water layer was discarded, and 200 μg. of 3-monoiodothyronine in 1 ml. of 0.1 *N* KOH added. After two hours the solution was dried in an air stream, the residue dissolved in ethanol-ammonia, and applied to Whatman #3 paper for descending chromatography in a butanol-6*N*-ammonia solvent sys-

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tem. Flanking strips carried iodide and 3:3′-Th markers. At the completion of the run the papers were dried and the marker strips stained for iodide with palladium chloride and for 3:3′-Th with diazotized sulfanilic acid or Ninhydrin. The zone corresponding to the 3:3′-Th markers was cut out and eluted with saline made alkaline with .1 *N* KOH. Yield was from 1 to 5 per cent. The final preparation was 80 to 90 per cent 3:3′-Th, as shown by chromatography in butanol-ammonia, butanol-acetic acid, and methanol-ammonium acetate solvent systems. Rf values corresponded closely to those reported by Gemmill (9).

Each patient was given from 10 to 50 μc. of I^{131}-labeled 3:3′-Th intravenously in 8 to 10 ml. of saline. An aliquot was saved for a standard, for chromatography, and for measurement of I^{127} content. Analysis indicated that each patient received from 20 to 40 μg. of unlabeled 3:3′-Th. Samples of urine and blood were collected as shown in the accompanying charts and tables. Two-ml. samples of serum were acidified to pH 2 with dilute H_2SO_4 and extracted three times with n-butanol in equilibrium with 5 per cent $Na_2S_2O_3$ in water. The pooled extracts were dried in an air stream and the residue dissolved in ethanol-ammonia and applied to Whatman #3 chromatography paper. Usually there was enough labeled iodine in the urine specimens to permit direct application of amounts to chromatography paper which would provide 2,000 to 5,000 counts per minute, as measured in a well-type scintillation crystal detector with pulse height analyzer. A few urine specimens had to be extracted with butanol in order to have an adequate amount of labeled iodine on the paper. Iodide and 3:3′-Th markers were always used. The stained chromatograms were cut in 1-cm. strips and measured for labeled iodine. Usually a butanol-2*N*-acetic acid solvent system was used, but occasional samples were run in a butanol-6*N*-ammonia solvent system. A small correction was always made for the labeled iodine found in zones of the paper which did not correspond to stained markers or in zones where recorded counts were in excess of 1 per cent of total counts per centimeter. This correction was usually less than 0.3 per cent of the total counts on the paper per centimeter.

RESULTS

The disappearance of labeled iodine from the blood and its appearance in the urine after administration of 3:3′-Th is shown in Figures 1 and 2. Samples were obtained from 3 patients for twenty-four to twenty-seven hours after administration. The concentration of labeled iodine in the serum fell rapidly for the first hour and then more slowly. Most of the labeled iodine had appeared in the urine by the end of twenty-four hours.

Only a small fraction of the labeled 3:3′-Th escaped unchanged into the urine (Table 1). From 8 to 30 per cent of the labeled iodine of the urine collected during the first hour after injection was present as 3:3′-Th, and later specimens showed much less. The total fraction of the dose of 3:3′-Th eliminated in the urine *as 3:3′Th* during the first four hours was less than 4.5 per cent, but during this time from 12 to 40 per cent of the administered dose of labeled iodine had appeared in the urine.

Chromatographic analysis of samples of serum indicated that labeled 3:3′-Th was rapidly degraded to iodide. The disappearance of 3:3′-Th from the serum is shown in Figure 3. Serial samples of blood were taken

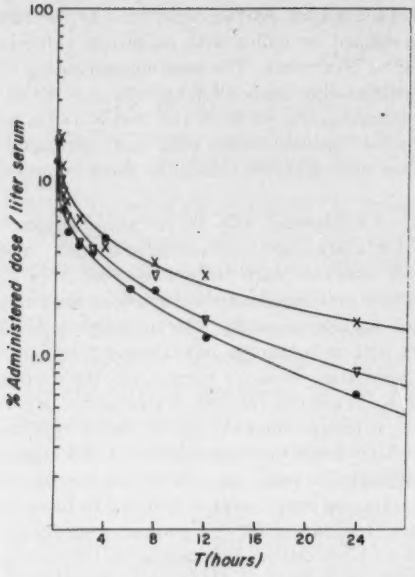

FIG. 1. The labeled iodine of serum after administration of I^{131}-labeled 3:3′-diiodothyronine. The abscissa denotes time in hours. The ordinate denotes per cent of dose per liter of serum plotted logarithmically. The administered dose contained approximately 80% of 3:3′-diiodothyronine.

during the first hour in 3 of the patients. Curves are drawn through points derived from these samples. Serum samples were taken at five and sixty minutes after injection in 5 of the other patients. These points are also plotted in Figure 3. There was a rapid initial fall in serum concentration, followed by a slower fall. The half-time of the slower phase was estimated to be of the order of an hour or less.

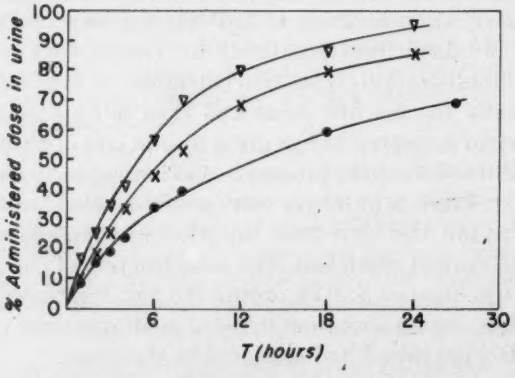

FIG. 2. The accumulation of labeled iodine in the urine after administration of I^{131}-labeled 3:3′-diiodothyronine.

TABLE 1

Patient No., Sex & Age	Diagnosis	3:3′-Th in injected dose	% Dose excreted			% I^{131} in urine as 3:3′-Th			% Administered 3:3′-Th excreted as 3:3′-Th		
			0–1 hr.	0–2 hrs.	0–4 hrs.	0–1 hr.	1–2 hrs.	2–4 hrs.	0–1 hr.	0–2 hrs.	0–4 hrs.
1 F 61	Thyrotoxicosis	84.7	7.0	13.9	22.0	30.0	13.1	4.9	2.5	3.5	4.0
2 M 70	Thyrotoxicosis	77.0	6.4	10.9	19.1	16.2	9.5	5.5	1.4	1.9	2.5
3 F 37	Thyrotoxicosis	80.1	15.4	22.9	38.1	14.4	4.5	0	2.8	3.2	3.2
4 F	Hypothyroidism; goiter	85.5	6.6	17.9	28.7	12.3	7.9	4.7	0.8	1.8	2.4
5 F 43	Acetic-acid ingestion	84.7	4.7	7.9	12.6	18.5	9.8	4.8	1.0	1.4	1.7
6 F 67	Cancer of tongue	90.3	11.1	19.3	34.6	17.0	4.6	3.7	2.1	2.5	3.1
7 F 70	Varicose ulcer	80.3	8.1	14.6	24.2	13.2	9.6	3.9	1.3	2.1	2.6
8 M 24	Myocardial infarct	80.3	14.7	22.3	38.0	13.2	4.5	0	2.4	2.9	2.9
9 F 45	Hodgkin's disease	90.2	10.0	18.4	33.1	8.2	4.0	3.9	0.9	1.3	1.9
10 M	Mental deficiency	91.7	16.4	24.3	33.4	17.5	7.1	4.0	3.1	3.7	4.5

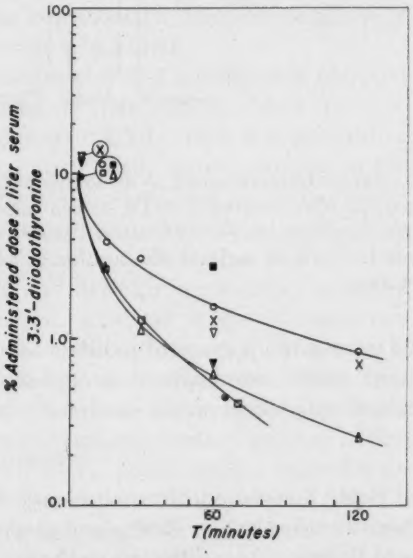

FIG. 3. The disappearance of 3:3′-diiodothyronine after intravenous administration. The abscissa denotes time in minutes. The ordinate denotes the concentration of labeled iodine present in the serum as 3:3′-diiodothyronine. All points are plotted, but serum samples were taken from only 3 of the patients at fifteen and thirty minutes. These have been connected by the curves as indicated.

Two unidentified zones of labeled iodine were found in some, but not all, samples of serum and urine. One of these appeared halfway between the iodide and the 3:3′-Th markers in the butanol-acetic acid solvent system (Fig. 4). It was present in 5 of 26 blood samples and in 4 of 8 patients. In 3 samples the amount of labeled iodine in this zone was small in proportion to that in the 3:3′-Th zone, but in the other 2 it accounted for a significant part of the labeled iodine on the chromatogram. A similar zone of labeled iodine was found in 8 of 32 samples of urine examined chromatographically from 4 of 10 patients. With 1 exception, the amount

of labeled iodine in this zone was less than in the 3:3′-Th zone. A second zone of labeled iodine was observed beyond the 3:3′-Th zone, but before the solvent front. This was found in 16 of 26 samples of serum from 6 of 8 patients. In 7 of the samples it accounted for more than 10 per cent of the labeled iodine on the chromatogram. The labeled iodine near the solvent front was not found in the urine samples. Two patients (Nos. 1 and

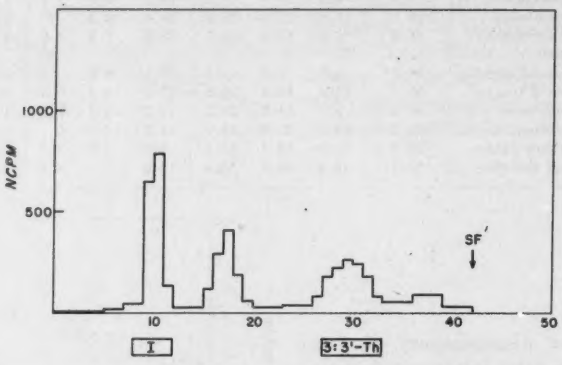

FIG. 4. Chromatogram to demonstrate the I^{131}-labeled substances of the serum in a patient who received 3:3′-Th sixteen minutes previously. The ordinate denotes net counts per minute per centimeter. The abscissa denotes cm. from the origin. SF: solvent front. The bars indicate the position of added markers. (Butanol-acetic acid solvent system.)

2) were given a gram of iodide as saturated solution of KI orally, half an hour before administration of 3:3′-Th. This appeared to have had no noteworthy effect on the results.

DISCUSSION

Both 3-monoiodothyronine and 3:3′-diiodothyronine (3:3′-Th) have been synthesized by Roche and associates (10) and by Gemmill, Anderson and Burger (9) by different methods. 3:3′-Th has been demonstrated in the thyroglobulin of the rat (6). Here it may constitute 25 per cent of the material usually attributed to thyroxine (which overlaps in most chromatographic solvent systems in common use), and similarly in the peripheral blood. The early appearance in the serum of 3:3′-Th after administration of I^{131} suggested to Roche *et al.* (8) that it was a secretion product of the gland rather than a substance formed peripherally by deiodination of triiodothyronine or thyroxine. Roche, Michel, Wolf and Etling (11) found that 3:3′-Th had antigoitrogenic activity which was almost equal to that of thyroxine, but Gemmill (12) found little or no antigoitrogenic effect.

After intraperitoneal injection in the rat, 3:3′-Th appears rapidly as iodide in the urine (13). Much of it appears in the bile of rats bearing biliary cannulae, indicating a large entero-hepatic circulation. Significant amounts of 3:3′-Th have been observed in the rat kidney after administration of I^{131}-labeled triiodothyronine (14).

After intravenous administration in man, I^{131}-labeled 3:3′-Th is rapidly degraded to iodide. Within five minutes after injection a significant fraction of the labeled iodine of the serum can be recovered as iodide, and after an hour usually less than 15 per cent of the labeled iodine is present as unchanged 3:3′-Th. One hour after injection, the highest concentration of unchanged 3:3′-Th among the 10 patients in this study was 2.7 per cent of the administered dose per liter of serum. Although the data are too meager to furnish precise values for degradation rates, a survey of Figure 3 suggests that labeled 3:3′-Th disappears very rapidly during the initial minutes after administration and then more slowly. The slower phase of disappearance had a half-time of the order of an hour.

The rapidity of disposal and degradation of 3:3′-Th is in sharp contrast to those of triiodothyronine or thyroxine. If the disposal of 3:3′-Th is as rapid as has been demonstrated in the present study, then it is difficult to see how an appreciable fraction of the iodine of the serum could be in the form of 3:3′-Th. The rapidity of degradation relative to thyroxine would necessitate secretion by the gland of very large amounts of 3:3′-Th in order to maintain a serum concentration comparable to that of thyroxine. It can be calculated that if thyroxine and 3:3′-Th are secreted at equal rates and the respective half-times in the blood are seven days and one hour, then the ratio of the 2 compounds in the serum at equilibrium will be 1/168, provided the volumes of distribution are the same. Thus if, as has been claimed (8), 25 per cent of the iodine of serum may be 3:3′-Th, then this compound would have to be the major secretion product of the gland. Since a large fraction of secreted 3:3′-Th would become immediately available for renal excretion as iodide, such large secretion rates of 3:3′-Th in proportion to thyroxine would of necessity result in rapid loss of labeled iodine from the gland and would not permit the observed normal half-time of labeled iodine, which is fifty to one hundred and fifty days. This estimate is not altered by the fact that the degradation rate of racemic 3:3′-Th is here compared to that of *l*-T_4. Not only does the degradation of *dl*-T_4 proceed at a rate similar to that of *l*-T_4 (15), but even if the contaminating *d*-3:3′-Th were degraded at a much slower rate than *l*-3:3′-Th, the observed rate in our series was much faster than that of *l*-T_4—both optical isomers of 3:3′-Th were almost entirely disposed of within two hours.

Appreciable amounts of 2 unidentified iodinated substances were ob-

served in some, but not all, of the patients after administration of 3:3′-Th. It is reasonable to suppose that these were pyruvate and lactate derivatives of 3:3′-Th. However, decarboxylation, glucuronide formation, or rupture of the phenoxy bridge may have occurred.

There was no distinctive pattern to the metabolism of 3:3′-Th by patients with Graves' disease when compared to patients without thyroid disease. Disposal of 3:3′-Th was similar to that in normal subjects in a young woman with familial goiter and hypothyroidism. Previously a normal degradation rate for diiodotyrosine had been found in this patient and the iodide in her thyroid was not eluted from the gland by administration of thiocyanate. The nature of her thyroid disease, therefore, was not determined.

SUMMARY

1. *dl*-3:3′-Diiodothyronine was given intravenously to 3 patients with Graves' disease, to 1 with familial goiter and hypothyroidism, and to 6 without thyroid disease. The rates of degradation and urinary excretion of the compound were similar in all subjects.

2. 3:3′-Diiodothyronine is rapidly degraded to iodide. After one hour, only a small fraction of the injected dose can be detected in the serum as the undegraded compound.

3. In some, but not all, patients 2 unidentified iodinated metabolic products were observed in the serum. One of these was also observed in the urine in small amounts.

4. By twenty-four hours most of the labeled iodine administered as I^{131}-labeled 3:3′-diiodothyronine appeared in the urine as inorganic iodide. Only a small percentage appeared in the urine as the parent compound.

5. The metabolic degradation of 3:3′-diiodothyronine is so rapid as to suggest that it can be present in the blood in only very small amounts relative to thyroxine, if at all.

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EFFECT OF THYROTROPIN ON THE PATTERN OF THYROID HORMONES IN PLASMA*

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THE data of some investigators would indicate that physical and emotional stress may influence various parameters of thyroid activity (1–6). The relatively prolonged latent period that precedes a demonstrable effect of thyroxine in both the experimental animal and man (7–11) has detracted attention from the role of the thyroid gland in the immediate metabolic response of the body to stress. As a result of the demonstration of the potent and rapidly acting thyroid hormone, 3:5:3′-*l*-triiodothyronine, in the serum of man and animals (12, 13), however, it seemed possible that this substance could serve as a so-called emergency-type hormone if it were elaborated in increased amounts in response to an acutely stressful situation. The functional relationship between the central nervous system, the anterior pituitary and the thyroid gland (2, 14, 15) suggests that such participation of the thyroid might be mediated through fluctuations in the secretion of thyrotropin. Because of these considerations, paper radiochromatographic techniques have been utilized to characterize the early changes in the pattern of iodinated compounds in plasma associated with the increase in the concentration of serum protein-bound iodine that follows the administration of thyrotropin to euthyroid and hyperthyroid individuals.

METHODS

The 4 euthyroid and 3 hyperthyroid patients who were the subjects of this study were given I^{131} in therapeutic doses ranging from 4 to 7 millicuries, and 10 to 15 millicuries, respectively. The euthyroid group (ages 32 to 62) consisted of 2 patients with advanced pulmonary emphysema and 2 with intractable cardiac failure associated with rheumatic valvular disease. The 3 thyrotoxic patients (ages 34 to 62) had typical clinical and laboratory evidence of thyrotoxicosis; their goiters were of the diffuse type, but proptosis and associated eye signs were not prominent in any.

Paper radiochromatograms were first obtained from extracts of plasma from blood sampled at six, twenty-four, forty-eight and occasionally as late as seventy-two hours after the administration of the therapeutic dose of radioiodide. A single dose of 10 to 20

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units of thyroid-stimulating hormone (TSH)[1] was then injected intramuscularly following the withdrawal of the last blood specimen (by which time the pattern of labelled iodinated compounds had become relatively stable). Serial paper radiochromatographic analyses were again performed on similar extracts of plasma from blood sampled at approximately three, six, twenty-four and forty-eight hours after the injection of TSH. The chromatographic pattern of the endogenously labeled iodinated substances of the plasma from blood drawn immediately prior to administration of TSH (forty-eight to seventy-two hours following the therapeutic dose of I^{131}) was then compared to the post-TSH pattern.

All blood specimens were oxalated and the plasma separated by centrifugation immediately following collection. An approximately 6-ml. sample of plasma was then flurried for ten minutes with 0.5 Gm. (dry weight) of an anion exchange resin.[2] This step was introduced in order to remove the excessive amounts of unbound radioiodide that frequently tend to obscure other adjacent small, but possibly significant, peaks of radioactivity. Preliminary studies have indicated that this resin removes little, if any, of the protein-bound radioactivity, and this would seem to be confirmed by the experience of others (16, 17).

A 4-ml. sample of the resin-treated plasma was extracted (without altering the pH) with a double volume, and then twice again with equal volumes, of n-butanol. Our data would corroborate those previously published (18), which indicate that 70 to 80 per cent of the protein-bound activity is extractable by this technique. It should be noted that thyroglobulin is not recovered by this procedure. The plasma extracts were usually prepared in duplicate for chromatographic fractionation in two different solvent systems. The pooled extracts were evaporated to dryness under a stream of air in a water bath at 50° C. The residue was then resuspended in 10 drops of ethanol-ammonia (9:1 mixture of 90 per cent ethanol and 27 per cent ammonium hydroxide), and approximately 10 to 20 micrograms each of powdered *l*-thyroxine[3], 3:5:3′-*l*-triiodothyronine[4], *l*-monoiodotyrosine[4], *l*-diiodotyrosine and potassium iodide added as carriers to lend assurance to the identification of the endogenously labeled compounds. Triiodothyroacetic acid[3] was also utilized as a carrier in a few instances. This ethanol-ammonia solution was subsequently applied to a 4–5 cm. line on a sheet of Whatman #1 filter paper, and single-dimension chromatographic separation carried out on the duplicate extracts in both an ascending n-butanol:2*N* NH_4OH system (1:1) and a descending tertiary amyl alcohol:2*N* NH_4OH system (1:1). The chromatograms were permitted to develop to a length of approximately 30 cm. over a 24- to 36-hour period, dried at room temperature and then sprayed with diazotized sulfanilic acid to locate the stable iodinated amino-acid carriers. Potassium iodide was identified by its color reaction with 1 per cent palladium chloride in a similar fashion.

The chromatograms were then divided horizontally into 0.5-cm strips and the radioactivity of each segment assayed individually in a well-type scintillation counter for a period of one minute. Counting rates that exceeded base-line activity by at least 50

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[1] The Thytropar (TSH) used in this study was kindly contributed by The Armour Laboratories, Kankakee, Illinois.

[2] Amberlite IRA-400 in the chloride phase (Rohm & Haas Co., Philadelphia, Pa.)

[3] Provided through the courtesy of Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

[4] Generously supplied by Dr. Rosalind Pitt-Rivers, National Institute for Medical Research, Mill Hill, London, England.

counts per minute on two or more successive strips were regarded as significant concentrations of radioactivity.

The concentration of radioactivity in the total plasma and in the protein-bound iodine (PBI^{131}) fractions was determined (16) in the sample withdrawn immediately prior to the administration of TSH, and in all of the serial specimens obtained following TSH injection. The protein-bound I^{127} concentration was determined (19) in most instances in serum sampled at these same time-intervals.

RESULTS

Previous studies in our laboratory have indicated that the major concentration of protein-bound radioactivity on the chromatograms of extracts of plasma obtained from euthyroid and hyperthyroid individuals following large tracer and therapeutic doses of I^{131} corresponds to the *l*-thyroxine area (20). In our experience it has been relatively difficult to demonstrate 3:5:3′-triiodothyronine in the plasma of man. This has been particularly true in euthyroid subjects, in whom it has been possible to detect this hormone in less than 10 per cent of those studied. This finding is somewhat in contrast to that in thyrotoxic individuals, in whom endogenously tagged and circulating 3:5:3′-triiodothyronine can be identified in approximately 30 per cent of cases (20).

The paper radiochromatograms of several euthyroid and hyperthyroid subjects in both this series of patients, and in a larger group previously studied (20) to whom TSH was not administered, have contained a peak of radioactivity at, or closely adjacent to, the juncture of the monoiodotyrosine and diiodotyrosine carrier areas (Fig. 3). It is our feeling that this activity does not represent either mono- or diiodotyrosine, since it does not coincide exactly with the carriers in any of the single-dimension solvent systems, nor does it adhere to the characteristic Rf values of either of these compounds in two-dimensional chromatographic systems (n-butanol:2N NH_4OH followed by methanol:0.2*N* ammonium acetate at 4° C.). Its identity, at present, is unknown to us, but neither the presence nor the magnitude of these peaks was seemingly affected by the administration of TSH. The occasional appearance of similar substances in other instances has been ascribed to the radiation effects on thyroid epithelium following large doses of radioiodide (21).

The serum concentration of protein-bound I^{127}, total radioactivity and protein-bound I^{131} (PBI^{131}) increased promptly (within zero to six hours) in all of the euthyroid and thyrotoxic subjects studied, following the intramuscular injection of 10 to 20 units of TSH. The magnitude of this response tended to be greater in the euthyroid subjects, but to occur more promptly in the patients with thyrotoxicosis—a phenomenon which has

been reported by other investigators (22, 23). The increment in the concentration of protein-bound I^{127} following TSH was in the range of 40 to 100 per cent of the control values, whereas the PBI^{131} moiety increased two- to fourfold over the pre-TSH levels.

A. *Paper radiochromatographic studies before and after administration of TSH to euthyroid subjects*

The pattern of response following the administration of TSH to the euthyroid individuals was similar in 3 of 4 instances (Fig. 1). The uppermost chromatogram in the figure represents the distribution plot of radioactivity along the length of the paper strip, obtained forty-eight hours following a therapeutic dose of I^{131} and immediately prior to the intramuscular injection of thyroid-stimulating hormone (TSH). This particular chromatogram served as the "control" pattern to which the post-TSH results were compared in all patients. The second and third chromatograms depict the I^{131}-labeled plasma components at various time-intervals subsequent to administration of TSH. An increase in radioactivity occurred promptly in the thyroxine area alone following TSH, as indicated by the increased height and width of the thyroxine curves in these representative chromatograms. This cannot be entirely appreciated from the figure, since the tops of the thyroxine peaks have been cut off for the convenience of illustration. Total thyroxine radioactivity following TSH was usually two to three times greater than before administration of TSH. No significant activity was apparent in the 3:5:3′-triiodothyronine area either before or after TSH administration.

The remaining euthyroid subject demonstrated a striking concentration of radioactivity that appeared in the 3:5:3′-triiodothyronine area between twenty-four and forty-one hours following administration of TSH (Fig. 2). This peak, however, was noted in only the tertiary amyl alcohol:2*N* NH_4OH system and not in the n-butanol:2*N* NH_4OH solvent system, and its identity with that of triiodothyronine is open to question. This type of discrepancy emphasized again the importance of studying a compound in more than one chromatographic system before being relatively certain of its identity. The nature of this peak of activity, which appeared in only one of the two different fractionation methods employed, is unclear to us.

Therefore, in this group of 4 euthyroid persons, the injection of TSH was uniformly accompanied by an elevation in the plasma concentration of I^{131}-labeled thyroxine. These data do not indicate, however, that the circulating level of tagged triiodothyronine, or any other iodinated amino acid, increased significantly following the administration of TSH to euthyroid subjects.

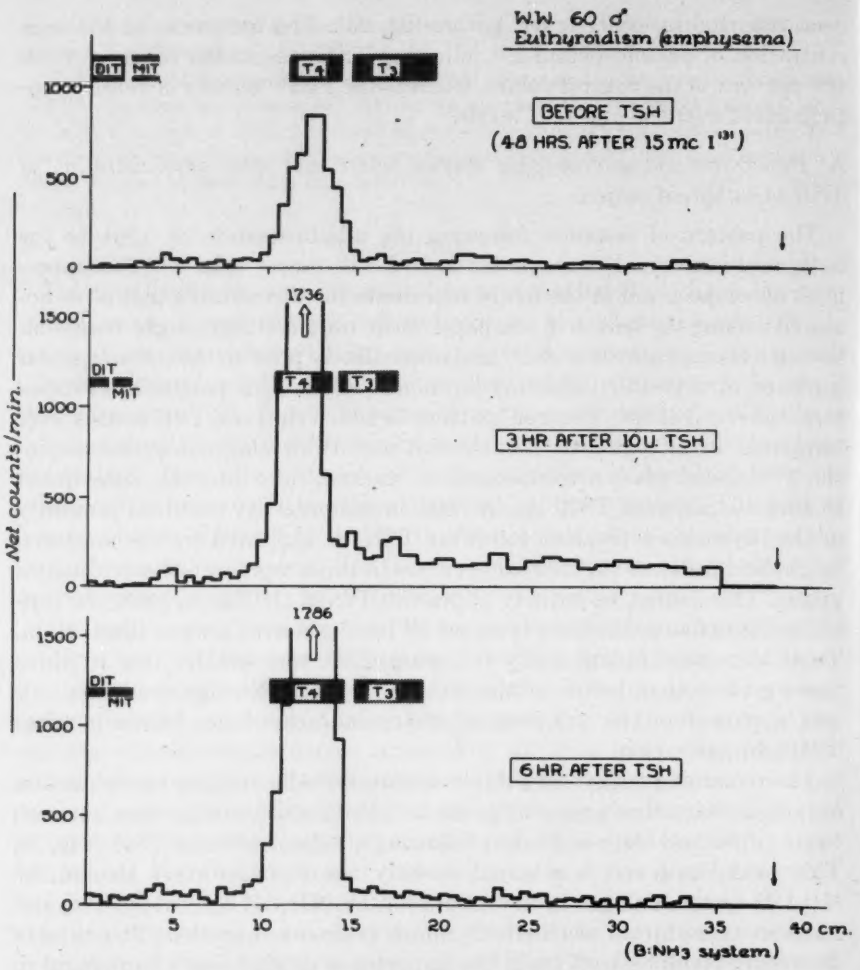

FIG. 1. The endogenously labeled I^{131}-components in n-butanol extracts of the plasma of an euthyroid subject (pulmonary emphysema) prior to and following the intramuscular injection of 10 units of TSH. The control paper radiochromatogram is that of the extract of plasma sampled at forty-eight hours after the administration of 15 millicuries of I^{131}. The 3-hour and 6-hour chromatograms are representative of the serial studies performed at frequent intervals, one to forty-eight hours following TSH. The abscissa is recorded in centimeters from the origin (extreme left) of the paper radiochromatograms developed in an n-butanol:2*N* NH_4OH solvent system. The ordinate is in net counts per minute per 0.5 cm. of the paper strip, and the numerical value at the apex of the zone of activity coinciding with the *l*-thyroxine area represents the maximum number of net counts per minute, since the entire peak could not be conveniently included in these diagrams.

B. *Paper radiochromatographic studies before and after administration of TSH to hyperthyroid subjects*

In each of the chromatograms for the 3 thyrotoxic patients there was a prompt increase in the concentration of radioactivity in the thyroxine area following the injection of TSH, as shown in the representative graph selected for illustration in Figure 3. Again, the total thyroxine radioactivity after TSH was two to three times more than the control value. Definite, but transient, elevations in the counting rate localized to the site of carrier 3:5:3′-triiodothyronine were also noted in all of the subjects in this group. This apparent increase in the concentration of circulating triiodothyronine occurred within three to six hours after administration of TSH. However, it had waned by twenty-four hours, at which time the chromatographic pattern had returned to its pre-TSH outline, except for the persistent elevation in the concentration of radioactivity of the endogenously labeled thyroxine.

No attempt was made to calculate or compare the pre-TSH and post-TSH triiodothyronine/thyroxine ratios expressed as the total number of counts per minute included under each of the respective peaks. Because of factors such as diffuse base-line activity, the occasional necessity for arbitrarily delineating the extent of the peaks along the abscissa, and the relatively low concentration of tagged triiodothyronine as compared to the large amounts of labeled thyroxine, such ratios would have had little numerical significance.

Thus, the hyperthyroid patients resembled the euthyroid subjects in that the major portion of the increment in protein-bound radioactivity following the injection of TSH could be ascribed to an increase in the plasma concentration of endogenously labeled and circulating thyroxine. In contrast to the euthyroid subjects, however, each of the thyrotoxic patients exhibited an early, but transient, elevation of small magnitude in the plasma concentration of tagged 3:5:3′-triiodothyronine.

C. *Studies of other iodothyronines and the acetic-acid analogues of thyroxine and triiodothyronine in plasma*

Recent chromatographic analyses of rat plasma following the administration of radioiodide have led to the characterization of 2 new iodinated constituents, namely 3:3′-diiodothyronine and 3:3′:5′-triiodothyronine

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MIT = *l*-monoiodotyrosine; DIT = *l*-diiodotyrosine; T_3 = 3:5:3′-*l*-triiodothyronine; T_4 = *l*-thyroxine. The arrow at the extreme right of the abscissa indicates the solvent front.

The carriers were located as described in the text. The peak of radioactivity in each chromatogram coincides with thyroxine; no definite concentration of I^{131}-labeled 3:5:3′-triiodothyronine is apparent.

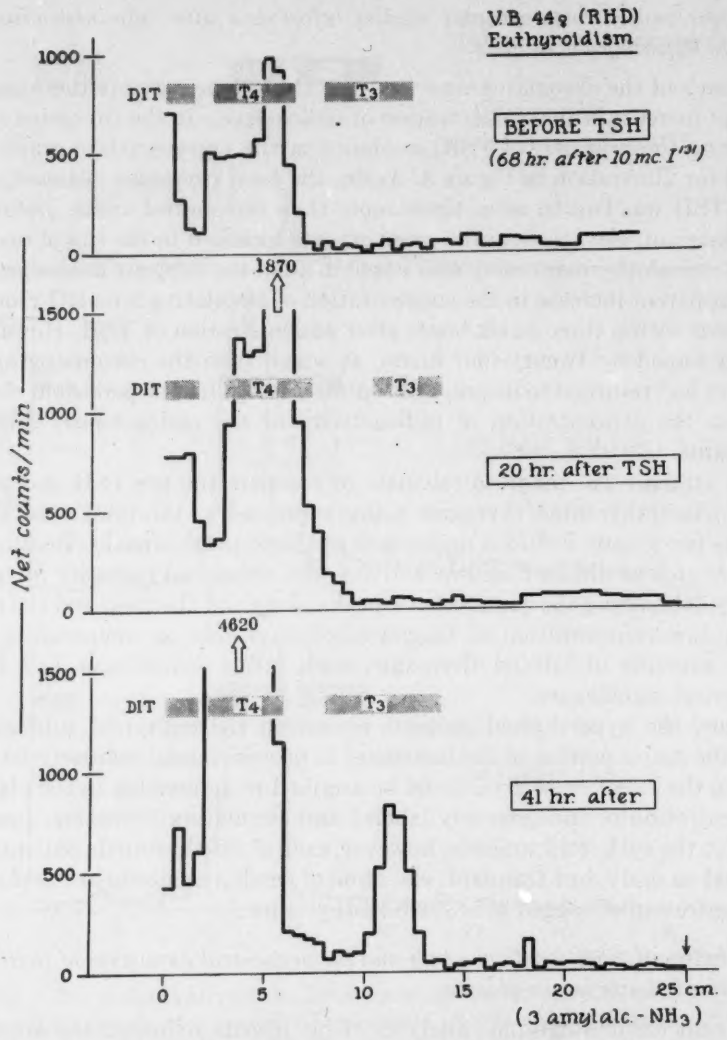

FIG. 2. Paper radiochromatograms of n-butanol extracts of the plasma of an euthyroid subject (rheumatic valvular disease), in whom there was an apparent increase in the concentration of labeled, circulating 3:5:3′-*l*-triiodothyronine between twenty- and forty-one hours following the intramuscular administration of 10 units of TSH. This increased activity, however, was demonstrated in the extracts developed with a tertiary amyl alcohol:2*N* NH_4OH solvent system only, and not with the *n*-butanol:2*N* NH_4OH separation of duplicate extracts. It is highly doubtful, therefore, that this peak represents triiodothyronine; its exact identity is unknown. The construction of the graphs is the same as in Figure 1.

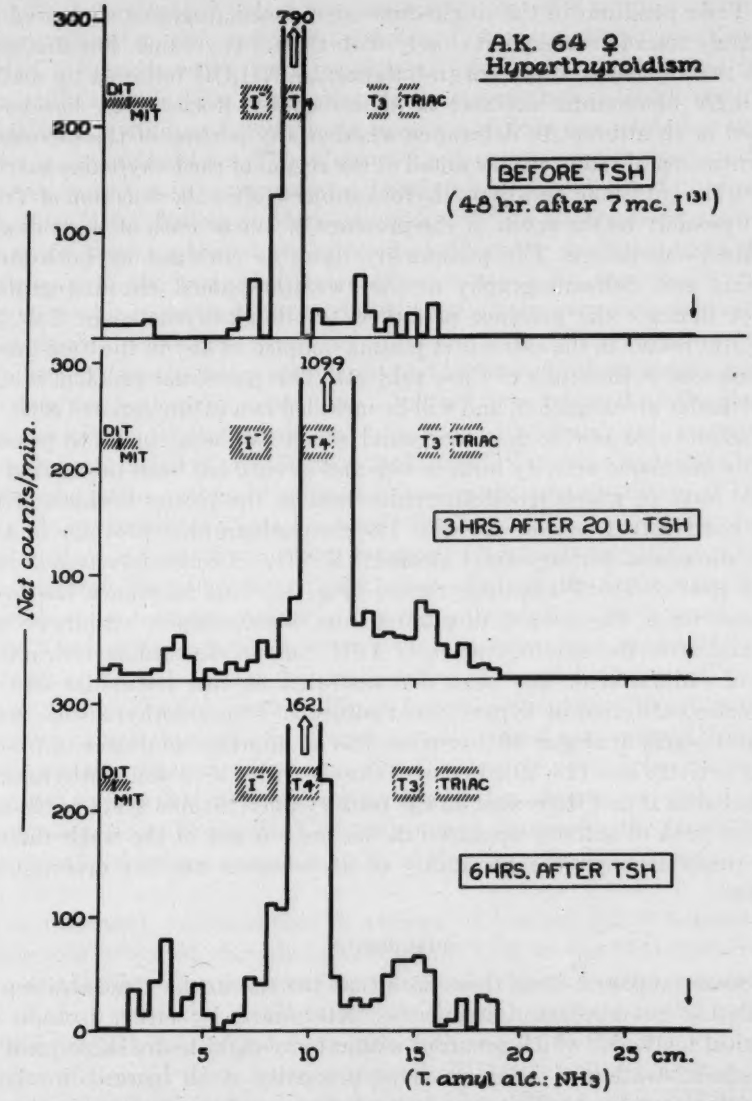

FIG. 3. Paper radiochromatographic fractionation (tertiary amyl alcohol:2*N* NH_4OH solvent system) of n-butanol extracts of the plasma of a patient with thyrotoxicosis, before and after the intramuscular injection of 20 units of TSH. The prompt rise in activity in the 3:5:3′-*l*-triiodothyronine area is apparent. In the 24-hour chromatogram (not shown here) there was no demonstrable triiodothyronine activity. TRIAC = triiodothyroacetic acid.

(24). Their positions in the single-dimension chromatograms employed in this study tend to correspond closely with that of thyroxine. For this reason, a two-dimensional system (n-butanol:2*N* NH_4OH followed by methanol:0.2*N* ammonium acetate) as described by Roche (24), has been utilized in an attempt to determine whether any portion of the increased concentration of radioactivity noted in the region of the *l*-thyroxine carrier in both the euthyroid and hyperthyroid subjects after the injection of TSH might possibly be the result of the presence of one or both of these newly described substances. The preliminary data, as obtained by both strip counting and radioautography of the two-dimensional chromatograms, do not indicate the presence of either 3:3′-diiodothyronine or 3:3′:5′-triiodothyronine in the extracts of plasma sampled at any of the time-intervals selected in the study of these subjects. This particular problem is currently under investigation, and will be included in a future report (20).

Triiodothyroacetic acid, a compound which has been shown to possess definite metabolic activity both *in vivo* and *in vitro* has been postulated to be the form in which triiodothyronine acts in the tissues because of its very short latent period (25–27). Its chromatographic position in the single-dimension tertiary amyl alcohol:2*N* NH_4OH solvent system is just above that of 3:5:3′-triiodothyronine (Fig. 3). This substance has been searched for in the extracts of plasma from blood samples withdrawn before and after the administration of TSH, but no discernible concentration of radioactivity has been demonstrated at this particular site in either the euthyroid or hyperthyroid subjects. Tetraiodothyroacetic acid, the acetic-acid analogue of thyroxine, has an appreciable degree of physiologic activity also (28, 29). It moves closely with 3:5:3′-triiodothyronine, but between it and thyroxine, in the tertiary amyl alcohol system. No significant peak of activity appeared in this area in any of the single-dimension runs, although the possibility of its presence was not investigated further.

DISCUSSION

It seems apparent from these data that the rise in the concentration of circulating protein-bound iodine (as determined by either isotopic or chemical methods) which occurred within forty-eight hours subsequent to the administration of TSH consisted primarily of an increase in the *l*-thyroxine component. This was particularly true of the euthyroid subjects studied, but slight, transient rises in the plasma concentration of 3:5:3′-triiodothyronine were noted also in the thyrotoxic patients.

It would appear that triiodothyronine—at least in the euthyroid person—does not serve as an emergency-type hormone insofar as stimulation by exogenous TSH can be utilized to simulate the mechanism involved in the

interplay between the central nervous system, anterior pituitary and the thyroid gland in the response to stress. Chromatographic analyses of labeled iodinated amino acids in thyroid vein blood of euthyroid dogs have indicated that the heightened PBI^{131} concentration which occurred within twenty minutes following the injection of TSH was related to an increase in thyroxine alone. The infusion of epinephrine and norepinephrine, however, resulted in increments of both thyroxine and triiodothyronine (30). The latter finding in dogs coupled with the observations made in this study suggests that if the thyroid gland of the normal subject does participate in the body's acute reaction to stress by secreting triiodothyronine, this response is not mediated through the increased elaboration of pituitary thyrotropin.

Failure to demonstrate a specific thyroid hormone in the general circulation does not necessarily imply that it has not been released by the gland. It is conceivable that a small, but physiologically significant, increment in the concentration of circulating 3:5:3′-triiodothyronine may have been undetected in the euthyroid subjects following the administration of TSH, using the extraction and assay methods described. The methods may have been inadequate for this purpose because of physical decay due to the time required for processing, the dilution effect of the large volume of plasma, or the transient nature of the rise in labeled triiodothyronine activity. The latter phenomenon seems remote in spite of the reduced affinity of serum alpha globulin for triiodothyronine (31) and the relatively rapid rate at which triiodothyronine diffuses from the circulation in contrast to thyroxine, since the half-life of exogenously tagged and infused triiodothyronine is on the order of 2.7 days (32). The high level of biologic activity possessed by 3:5:3′-*l*-triiodothyronine (and other thyronines such as triiodothyroacetic acid) implies that only minor changes in the serum concentration of these substances may be of considerable clinical significance (33).

The increased concentration in plasma of labeled 3:5:3′-triiodothyronine that attended the administration of TSH to the thyrotoxic subjects in this study may represent an additional difference in the responsiveness of the hyperactive gland to thyrotropin, as compared to the normal thyroid (22, 23). Available information with respect to the chemical composition of thyroid tissue in untreated exophthalmic goiter indicates that the mean distribution of iodine among the known and separable iodine-containing constituents is not essentially different from that in normal thyroid tissue, and that the total iodine content of the former is only slightly less than in the normal gland (34). Consequently, this seemingly unique reaction of the hyperactive gland to stimulation with TSH would appear to be related to an innate difference in response rather than to the

amount or type of iodinated amino acids present in the gland at the time of injection. Thyrotropin has been demonstrated to have an effect on a variety of functions related to the synthesis of thyroid hormone as well as to its release (35). A possible and likely explanation for the results noted here with exogenous TSH stimulation would be an early, but short-lived, effect of TSH on the discharge of both preformed 3:5:3′-triiodothyronine and thyroxine from the hyperactive gland, in contrast to the release of increased amounts of thyroxine alone, from the normal thyroid. This increment in the plasma radioactivity of tagged 3:5:3′-triiodothyronine occurred so promptly after administration of TSH that it is difficult to ascribe it solely to an increased rate of triiodothyronine synthesis by the hyperactive gland, although radioiodide transfer among the iodinated amino acids has been reported to occur more rapidly in goitrous glands than in normal ones (34). The fact that 3:5:3′-triiodothyronine is more easily demonstrable in the plasma of thyrotoxic patients than of euthyroid subjects (20) would indicate an increased circulating level of this hormone in hyperthyroidism. This situation may denote that more triiodothyronine is being released into the periphery in patients with diffuse toxic goiter, and for this reason is more readily elaborated by the hyperactive gland than by the normally functioning thyroid in response to stimulation with TSH.

It was impossible in the current study, as in other previous experiments utilizing similar chromatographic techniques, to be certain that the labeled thyroxine and triiodothyronine of plasma had the same specific activity in both the euthyroid and hyperthyroid subjects. For this reason, one cannot assume that there is an absolute correlation between an increase in the levels of tagged triiodothyronine or thyroxine and changes in the chemical levels of these hormones. It is thus possible, although unlikely, that the increment in triiodothyronine concentration that follows administration of TSH to hyperthyroid subjects is related to the greater specific activity of this substance in thyrotoxic patients as compared to the euthyroid subjects. This particular problem cannot be solved at present, since specific chemical methods for the quantitative measurement of triiodothyronine and thyroxine are not available. This factor, in addition to the rate of release, vitally affects the level of the plasma PBI^{131} and any estimate of the absolute concentration of the constituents comprising this particular fraction.

The likelihood that the peripheral conversion of the large amounts of tagged thyroxine to labeled triiodothyronine (36, 37) accounted for the readily detectable levels of this latter substance in the plasma of thyrotoxic patients seems unlikely in view of the fact that the concentration of radioactivity in the plasma PBI^{131} moiety was higher in almost every instance

following the administration of TSH to the euthyroid subjects (because of the greater amounts of administered I^{131}), and yet no circulating triiodothyronine was demonstrable. Another possible factor of indeterminate importance in this regard is the recently postulated (38) adaptive thyroxine deiodinating enzyme system that is conditioned by the level of thyroxine in the tissues. This may be another explanation for the increased concentration of triiodothyronine in the plasma of hyperthyroid subjects, but its role in the observed response to TSH is difficult to evaluate at this time.

A recent report of the chromatographic analysis of labeled iodinated compounds in the lymphatic pathways leading from the thyroid gland suggests that this may be a more important site than the thyroid vein or peripheral blood in which to search for changes in the rate of secretion of thyroxine and other iodothyronines (39). Such a possibility may well be kept in mind in evaluating the effects of TSH reported in this study.

The response of the thyroid gland to stimulation with TSH has been found to be impaired following therapeutic doses of radioiodide, when evaluated by a variety of tracer techniques at remote intervals (eight weeks to several months) after the ionization effects have occurred (40, 41), and this contingency warrants consideration. Similar studies, however, performed as early as seven days after a therapeutic dose of I^{131} have indicated that the administration of TSH at this time is followed by thyroidal responses similar to those exhibited by untreated glands (42). It is unlikely that the significance of the findings reported in the present study has been modified by a radiation effect on thyroid epithelium, since thyrotropin was injected in each instance within seventy-two hours after the therapeutic dose of radioiodide.

SUMMARY

1. Paper radiochromatographic techniques have been utilized to identify the iodinated substances in plasma which account for the rise in the levels of protein-bound I^{127} and I^{131} that follows the administration of thyroid-stimulating hormone (TSH).

2. In 3 of the 4 euthyroid subjects studied, the injection of TSH was followed by an increase in the level of thyroxine alone. In each of the 3 thyrotoxic patients there were slight, transient increments in the concentrations of circulating and endogenously labeled 3:5:3′-triiodothyronine three to six hours following the administration of TSH, as well as marked increases in the concentration of tagged thyroxine.

3. Preliminary studies utilizing two-dimensional chromatographic systems in the fractionation of the plasma of euthyroid and hyperthyroid subjects failed to reveal any detectable concentrations of I^{131}-labeled 3:3′-

diiodothyronine or 3:3′:5′-triiodothyronine, before or after administration of TSH. Single-dimension chromatographic procedures were also unsuccessful in demonstrating triiodothyroacetic acid in the strips specifically examined for this substance.

Acknowledgments

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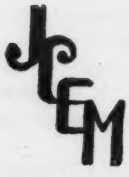

ADRENOCORTICAL FUNCTION IN MYXEDEMA*†

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THERE has been a prevailing concept that adrenocortical function is decreased in myxedema. This concept has been based mainly upon the decreased urinary excretion of 17-ketosteroids (1–12) in almost all cases of myxedema, the decreased excretion of formaldehydogenic (13), 17-ketogenic (12), 11-oxy (8, 14, 15) and 17-hydroxy corticosteroids (11) in many, and the failure of adrenocorticotropin (ACTH) to produce a fall in circulating eosinophils (7, 10, 15) in at least some of the cases studied. However, the reported information is somewhat conflicting, both as to findings and to interpretation.

Previous studies from our laboratory (16) have shown that in myxedema there is delayed diuretic response to a water load. This is not correctable by cortisone, but is corrected by thyroid therapy—indicating that this defect is not due to adrenal insufficiency.

With the development of methods for studying adrenocortical function and functional capacity or reserve more specifically and directly, the following investigation was undertaken.

MATERIALS AND METHODS

Thirteen patients (3 men, 5 premenopausal women, and 5 postmenopausal women) with primary untreated myxedema were studied (Table 1). The myxedema was idiopathic in 10, post-thyroidectomy in 2, and post-I^{131} irradiation in 1. The circulating plasma levels of free 17-hydroxycorticosteroids before and following stimulation with ACTH were used as the criterion of adrenocortical function and functional capacity or reserve. Two patients underwent 2 studies each, and in 3 patients studies were repeated after therapy with *l*-triiodothyronine.[1] Twelve of the 13 subjects were hospitalized and all 13 were considered to be in a "steady state" at the time of the studies. They were in a fasting state during the tests, which were begun between 7 and 8 A.M. After a sample of blood was drawn (using heparin for anticoagulation), a dose of 25 I.U. of ACTH in 500 cc. of 5 per cent glucose in distilled water was given intravenously at a constant rate over a four-hour period. At the end of the infusion, a second sample of blood was drawn. The blood was centrifuged and the plasma separated within an hour after venipuncture. The plasma was refrigerated or frozen until processed. All determinations

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[1] Kindly furnished by Dr. Arthur Heming of Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

were made on duplicate plasma samples, using Wallace, Christy and Jailer's (17) modification of the Silber-Porter (18) method.

The 24-hour urinary excretion of 17-ketosteroids was determined in 9 patients, multiple determinations being made in most cases. The specimens were analysed by a modification of the method of Callow, Callow and Emmons (19).

The biologic half-time of intravenously administered hydrocortisone[2] was determined in 3 of the patients, and in 3 normal subjects for controls. With the patient in the fasting state, a dose of 100 mg. of hydrocortisone in 500 cc. of 5 per cent glucose in distilled

TABLE 1. DATA ON 13 CASES OF MYXEDEMA

Patient, Age & Sex	Approx. duration of symptoms (yrs.)	Thyroidal 24-hr. I^{131} uptake (%)	BMR (%)	Serum cholesterol (mg./100 cc.)	Urinary 17-KS (mg./24 hrs.)	Plasma 17-OH-CS (μg./100 cc.)	
						Pre-ACTH	Post-ACTH
R.E., 57, M	10	7.4	−34	335	6.9	16.8	29.4
W.T., 43, M	2–3	6.4	−23	702		18.7	39.4
W.C., 58, M	13	7.9	−39	780	3.6	31.0	71.6
						25.8	65.2
L.W., 34, F	6		−44	274	1.2	25.9	40.7
A.T., 38, F	2	9.2	−29	454		29.5	56.6
M.S., 20, F	4		−33	492		11.5	44.9
J.L., 32, F	9	9.8	−28	466	4.0	14.8	43.6
O.S., 31, F	9	7.5	−39	512	3.6	17.9	46.5
M.P., 49, F	4	14.4	−38	600	2.4	26.2	56.4
						31.2	43.8
B.S., 65, F	8	13.7	−28	504	4.5	27.0	58.8
B.H., 64, F	4	10.3		424	3.0	9.2	27.2
L.Q., 55, F	6	13.6	−35	405		27.7	47.9
A.B., 62, F	8	15.9	−42	546	6.3	5.9	37.6

water was administered intravenously in a thirty-minute period. Blood specimens were drawn at one, two, four and six hours after the beginning of the infusion and analyzed for plasma 17-hydroxycorticosteroid content. By plotting the values against time on semi-log paper, a straight line was obtained, allowing for extrapolation to zero time. From these data the time was determined at which the 17-hydroxycorticosteroid value was half of the extrapolated value at zero time.

RESULTS

In Figure 1 each solid line represents one ACTH stimulation test, connecting the pre-ACTH with the post-ACTH plasma 17-hydroxycortico-

[2] Kindly furnished by Dr. Neal O'Donovan of the Upjohn Co., Kalamazoo, Michigan.

steroid (17-OH-CS) level. The stippled band represents the normal range of plasma 17-OH-CS concentration, both before ACTH (4 to 34 μg. per 100 cc.) and after ACTH (35 to 54 μg. per 100 cc.). For comparison, lines representing the average values found in hyperadrenocorticism, hypopituitarism, and primary adrenocortical insufficiency are also shown.

The pre-ACTH plasma 17-hydroxycorticosteroid levels were within normal limits in all the myxedema patients. The post-ACTH levels were within normal limits in 8, below normal in 2, and above normal in 5 tests

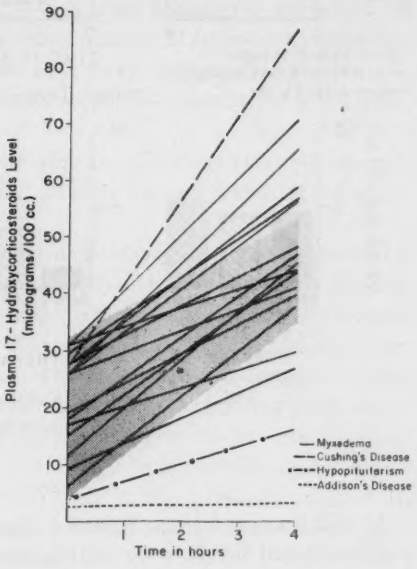

FIG. 1. Response of plasma 17-hydroxycorticosteroid levels to exogenous ACTH. (For explanation of graph, see text under Results.)

(Table 1 and Fig. 1). The 2 highest levels were both obtained on the same patient.

The biologic half-time of intravenously administered hydrocortisone was 3.3, 2.8 and 2.8 hours for the 3 myxedema patients, with an average of 3.0 hours. In the control group, the half-time was 2.2, 2.3 and 2.3 hours, with an average of 2.3 hours. In the single patient in whom the study was repeated after seven months of therapy with *l*-triiodothyronine, the biologic half-time was 1.5 hours, as compared with 2.8 hours before therapy.

Among the 9 patients in whom urinary 17-ketosteroids were measured prior to therapy, 8 had low excretions and 1 had a low-normal excretion (Table 1).

Studies of plasma 17-hydroxycorticosteroids and urinary 17-ketosteroids in 3 patients after therapy with *l*-triiodothyronine are shown in Figure 2.

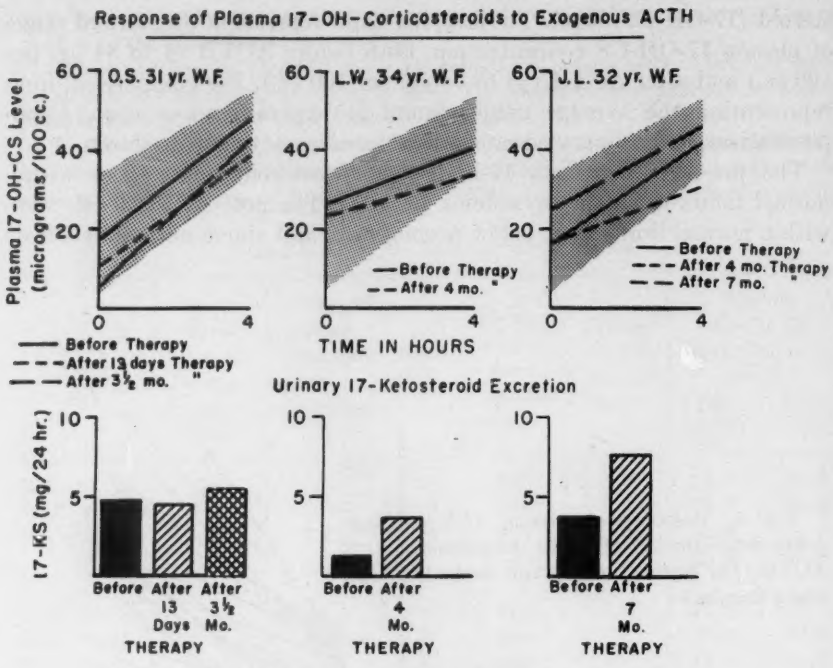

FIG. 2. Studies before and after therapy with *l*-triiodothyronine in 3 patients.

DISCUSSION

It will be noted from Figure 1 that the pre-ACTH plasma 17-hydroxycorticosteroid levels were within normal limits in all the myxedema patients. Values similar to those in normal fasting subjects were found in some of the patients studied by Perkoff *et al.* (20) and in the patients studied by Levin and Daughaday (11). The post-ACTH levels were within normal limits in 8 cases, below normal in 2, and above normal in 5. The 2 subnormal values were not, however, in the range which we have found in hypopituitarism or primary adrenal insufficiency. The 2 highest values were obtained in the same patient.

At attempt was made to explain this variation in post-ACTH levels of plasma 17-hydroxycorticosteroids. There was no correlation between the 17-hydroxycorticosteroid level and the age or sex of the patient or the duration or severity of the myxedema (Table 1).

In the patient with the 2 highest post-ACTH levels, the concentration of blood urea was 57 mg. per 100 cc. However, in 1 patient with a normal re-

sponse to ACTH and in 1 patient with a low response the blood urea values were essentially the same.

It would be expected that the state of renal function would have less effect on plasma 17-OH-CS values than on urinary levels. Peterson, Wyngaarden *et al.* (21) pointed out that, since less than 1 per cent of administered hydrocortisone is excreted in the unaltered free form, no accumulation of free hydrocortisone in the body would likely result from renal failure.

In the 3 patients on whom liver function studies were carried out, no significant abnormalities were found. It has been shown by Brown *et al.* (22) that, although the disappearance from plasma of infused hydrocortisone is slower than normal in patients with liver disease, the plasma 17-hydroxycorticosteroid levels before and after ACTH stimulation are within normal limits.

It would, therefore, appear that the plasma 17-hydroxycorticosteroid levels in myxedema are not materially altered by any existing renal or hepatic dysfunction.

Levin and Daughaday (11) found that the disappearance from plasma of infused hydrocortisone was delayed in patients with myxedema. A similar delay was observed in 2 of 3 myxedema patients studied by Peterson, Wyngaarden *et al.* (21). In our study also, the biologic half-time of intravenously administered hydrocortisone was found to be longer in the myxedema patients than in the normal subjects, but our series is very small. These studies suggest a reduced rate of hydrocortisone metabolism in myxedema. Peterson and Wyngaarden (23), using isotopic techniques, observed a reduced turnover of hydrocortisone in the presence of normal plasma levels in myxedema; this finding is also indicative of a reduced rate of hydrocortisone metabolism in myxedema.

Studies were repeated on 3 of the patients after therapy with *l*-triiodothyronine (Fig. 2). In Patient O.S., the plasma 17-hydroxycorticosteroid levels thirteen days and again three and a half months after therapy was begun were slightly lower than before therapy, but still within normal limits. The same phenomenon occurred in Patient L.W. after four months of therapy, the post-ACTH level of 17-OH-CS being just below the lower limit of normal. Patient J.L. responded similarly after four months of therapy, but after seven months the plasma 17-OH-CS levels before and after ACTH were slightly greater than before therapy. However, since there is normally a rather wide variation in 17-OH-CS levels in the same patient from day to day, and since all levels were essentially within normal limits, these differences are probably not significant.

Only in Patient J.L. (Fig. 2) were the foregoing determinations made be-

fore and after seven months of therapy with *l*-triiodothyronine. In addition, the biologic half-time of intravenously administered hydrocortisone was measured. The plasma 17-hydroxycorticosteroid levels were not significantly changed after therapy. The 17-ketosteroid excretion rose to within normal limits. The biologic half-time of intravenously administered hydrocortisone decreased from 2.8 hours before therapy to 1.5 hours after therapy. This post-therapy biologic half-time was even less than the average of 2.3 hours found in our normal subjects; the significance is not clear and the patient is not available for further study.

SUMMARY

Results of studies made on 13 patients with primary myxedema suggest that there may be a diminished rate of metabolism of adrenal corticosteroids in this disease.

Before infusion of ACTH the plasma 17-hydroxycorticosteroid levels were within normal limits in all 13 patients, indicating that responsive adrenals were being adequately stimulated by endogenous ACTH and that pituitary adrenocorticotropic activity was therefore adequate. These normal pre-ACTH levels in all patients and the adequate response to exogenous ACTH stimulation in all but 2 suggest that adrenocortical function and functional capacity or reserve, as determined by the ACTH test, were within essentially normal limits.

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PATHOLOGIC CHANGES IN ADULT MYXEDEMA: SURVEY OF 10 NECROPSIES*†

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INTRODUCTION

THE pathology of adult myxedema as recorded in the literature is based upon the findings of relatively few autopsies. The opportunity has seldom been presented to study at necropsy either untreated or inadequately treated cases. The present study was initiated because of the availability of 10 such cases at the Wayne County General Hospital in a 15-year period. Typical clinical features of the myxedematous state were present in all.

The first clinical recognition of myxedema has been attributed to Sir William Gull (1) in 1873. The first autopsy in a case of myxedema was reported in 1877 by Sir William Ord (2). The term "myxedema" was proposed by Ord, because the chief feature appeared to be a mucin-yielding edema involving chiefly the connective tissue. Subsequent authors described the similarity of spontaneous myxedema, cachexia strumipriva (post-thyroidectomy myxedema) and cretinism. Loss of thyroid function later became recognized as the common denominator of these states. The Report of a Committee of the Clinical Society of London (3) published in 1888 included the data on 20 autopsies of cases of myxedema. This publication is the classic source of reference for the pathology of myxedema. Since that time, 10 autopsies have been reported by Bastenie (4), and 9 by Means (5). A few single case reports have appeared (6–14). Two cases were studied by histochemical methods (6, 7).

METHODS AND MATERIAL

The 10 cases of myxedema studied had been clearly diagnosed during life by characteristic symptoms, physical signs and laboratory tests. There were 6 males and 4 females in the group, and the ages ranged from 62 to 78 years. Two of the women had had a thyroidectomy. One man had evidence of pituitary myxedema (panhypopituitarism, due to pituitary necrosis). The remaining 7 patients were considered examples of primary spontaneous adult myxedema. Two of the latter group received no thyroid therapy, 2 of the others were treated with thyroid for only a few days prior to death, and 6 received small doses of thyroid for irregular periods.

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Clinical records and autopsy protocols were reviewed and collated. In each case representative tissue specimens had been fixed in 10 per cent formalin for at least twenty-four hours, then embedded in paraffin. Tissue sections were cut 6 microns in thickness for microscopic examination. In each case embedded tissue specimens were kept in reserve, and new microscopic slides were made from these older stored specimens. The staining techniques employed were standard hematoxylin and eosin, periodic acid-Schiff (PAS) counterstained with hematoxylin (15), 1 per cent aqueous toluidine blue applied for thirty seconds or less, Mayer's mucicarmine (16), and phosphotungstic acid-hematoxylin (PTAH) (17). Histologic and histochemical interpretations were made on the basis of this material.

Specimens obtained during routine autopsies on 3 patients having no evidence of endocrine abnormality were similarly examined and served as controls.

RESULTS

Gross anatomic findings are presented in Table 1.

Primary myxedema

The following microscopic features were common to the 9 examples of primary myxedema. (The single example of pituitary myxedema is discussed later.)

Thyroid. The parenchyma was completely replaced by dense, partly-hyalinized fibrous tissue in which were interspersed a few focal accumulations of lymphocytes and plasma cells (Fig. 1a). Surrounded by these cells were residual thyroid follicles that had undergone Hürthle-cell metaplasia and varying degrees of squamous metaplasia. Many degenerative epithelial forms with pyknotic nuclei were present. The remaining follicles were very small and often appeared as clumps of cells without lumens (Fig. 1b). Colloid, if present, stained poorly. In 4 glands (Cases 4, 5, 9 and 10) occasional multinucleated foreign-body type giant cells were present in these areas.

Pituitary. No histologic abnormality was observed. However, only single sections of the gland were available for study and differential counts of cell types were not performed. Small colloid-filled follicles were often seen in the anterior lobe.

Adrenals. Lipid depletion of the cells of the cortex was present to a minimal degree in all but 2 cases (Nos. 5 and 6). In 1 instance (Case 10) there was an ischemic infarct of the cortex, associated with focal areas of hemorrhage in the cortex and medulla due to old thrombosis with organization of the adrenal veins.

Ovaries. These glands were atrophic, fibrotic and almost entirely composed of corpora albicantia. Atrophic changes were also present in the breast and uterus.

Testes. In Case 4 there was marked atrophy of the testes with fibrous thickening of the basement membranes and decreased numbers of interstitial cells. The tubules were lined only by Sertoli cells. In Case 10 the microscopic structure was characterized by a minor degree of peritubular fibrosis and some decrease in spermatogenesis.

Brain. The cerebral cortex was atrophic in 5 cases (Nos. 1, 5, 6, 8 and 10). The cerebellum grossly appeared somewhat disproportionately enlarged and the ventricular space was increased in size in Case 1.

Skin. Some degree of hyperkeratosis and atrophy of the epithelial layer was detected in sections of skin from the anterior abdominal wall and lateral aspect of the ankle (Fig. 2a). In addition, homogeneous basophilic substance was deposited in the papillary and upper reticular layers of the dermis. This area also appeared metachromatic with tolui-

TABLE 1. SUMMARY OF ANATOMIC FINDINGS

Patient		Thyroid gland	Other endocrine glands	Heart	Arteries	Gastro-intestinal tract	Serous cavities	Associated conditions	Cause of death
No.	Sex & age								
1	M 79	30 Gm., pale and fibrotic	Pituitary normal. Testes 50 Gm. each	700 Gm., RVH* and LVH,* coronary sclerosis	Marked sclerosis of aorta, cerebral and medium-sized arteries	Atrophy of gastric mucosa. Cholelithiasis. Focal subserous hemorrhages of small intestine	Mild hydrothorax and mild ascites	Papillary atrophy, thickened and enlarged tongue. Thickened vocal cords. Hydroceles	Heart failure due to arteriosclerotic heart disease. Pulmonary embolism (coma)
2	M 62	15 Gm., light gray and fibrotic	No data	350 Gm., LVH, coronary sclerosis	Marked sclerosis of aorta. Dissecting aneurysm of thoraco-abdominal aorta with redissection into lumen	Normal	Marked hydrothorax and marked ascites	No data	Heart failure due to hypertensive and arteriosclerotic heart disease
3	M 65	7.4 Gm., light gray and fibrotic	Pituitary and testes normal. Adrenals 17 Gm.	Severe coronary sclerosis, old and recent infarcts	Marked sclerosis of cerebral arteries. Aneurysms of internal iliac arteries	Subserous hemorrhages and marked distention of sigmoid colon. Distention of remainder of digestive tract	Hydrothorax and marked ascites	Enlarged and thickened tongue. Clear mucinous fluid in knee joint. Hydroceles	Thrombosis of right coronary artery
4	M 73	20 Gm., atrophic	Pituitary normal. Adrenals 8 Gm. Testes atrophic	310 Gm., RVH and LVH, severe coronary sclerosis	Marked sclerosis of aorta and cerebral arteries	No data	Marked ascites	Massive recurrent ascites during life. Inguinal and ventral hernias	Hemorrhage from chronic peptic ulcer of lower esophagus
5	F 72	10 Gm., firm and atrophic	Pituitary normal. Adrenals 20 Gm., normal	475 Gm., LVH, coronary sclerosis	Marked sclerosis of aorta and cerebral arteries	Diverticulosis and marked distention of sigmoid colon. Mild distention of remainder of digestive tract. Surgical absence of gallbladder	Normal	Psychosis, clinical	Heart failure due to hypertensive and arteriosclerotic heart disease (coma)

TABLE 1—(*continued*)

Patient No.	Sex & age	Thyroid gland	Other endocrine glands	Heart	Arteries	Gastrointestinal tract	Serous cavities	Associated conditions	Cause of death
6	F 63	5 Gm., remnant (old thyroidectomy)	Pituitary normal. Adrenals 12 Gm., normal. Ovaries atrophic, fibrotic	660 Gm., LVH, recent coronary occlusion with infarct, rupture of myocardium and hemopericardium	Marked sclerosis of aorta and cerebral arteries	Distention of colon. Polyposis of stomach and colon	Normal	Psychosis, clinical	Coronary thrombosis, myocardial infarction with rupture and hemopericardium. Pulmonary embolism
7	M 73	20 Gm., multinodular	Marked atrophy of pituitary (3×5 mm.), adrenals and testes (26 Gm.)	490 Gm., LVH, coronary sclerosis, fibrinous pericarditis (uremic)	Marked sclerosis of aorta, mild sclerosis of cerebral arteries	Normal	Hydrothorax and ascites	Panhypopituitarism. Lues. Old cerebral infarcts	Uremia, due to pyelonephritic atrophy of kidney
8	F 65	22 Gm., left lobe only, remnant (old thyroidectomy)	Pituitary and adrenals normal. Ovaries atrophic and fibrotic	290 Gm., LVH, slight coronary sclerosis	Sclerosis of aorta	Normal	Normal	Marked rheumatoid arthritis. Old subdural hemorrhage	Bronchopneumonia and miliary tuberculosis (coma)
9	F 67	Small, pale and fibrotic	Adrenals 16 Gm. Ovaries atrophic and fibrotic	500 Gm., LVH, coronary sclerosis, small old infarct	Marked sclerosis of aorta, sclerosis of cerebral arteries	Marked distention of stomach and colon	Normal	Papillary atrophy and enlarged tongue. Clear mucinous fluid in knee joints. Old cerebral infarct	Bronchopneumonia
10	M 74	23 Gm., firm and fibrotic	Pituitary normal. Adrenals 17 Gm. Hemorrhagic infarction due to venous thrombosis. Testes normal	370 Gm., LVH, coronary sclerosis	Marked sclerosis of aorta, sclerosis of cerebral arteries	Marked atrophic gastritis. Cholelithiasis.	Normal	Tongue and vocal cords normal. Inguinal hernia. Hydrocele	Hemorrhagicinfarctions of adrenal cortices due to venous thrombosis. Chronic pneumonitis

* RVH =hypertrophy of right ventricle; LVH =hypertrophy of left ventricle.

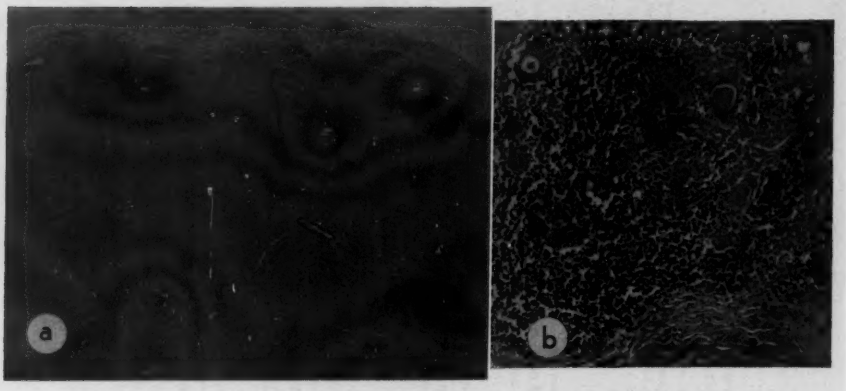

FIG. 1. (Case 2) **a.** Section through thyroid gland, showing atrophy and replacement fibrosis (hematoxylin and eosin; ×10). **b.** Residual focal area of degenerated follicular epithelium surrounded almost entirely by lymphocytes (hematoxylin and eosin; ×120).

dine blue and slightly pink in 2 instances with mucicarmine. There was also a slight increase in PAS-positive material in 2 cases. In Case 1 an elastica stain demonstrated very few elastic fibers in this area. The collagen fibers of the upper dermis were loosened and fibrillar. These changes indicated the deposition of mucoid material in the ground substance.

Structures of the head and neck. In the tongue there was a moderate increase in basophilic ground substance of the subepithelial connective tissue in the available sections studied (Cases 1, 9 and 10). In Case 9 this layer appeared faintly metachromatic with toluidine blue, faintly pink with mucicarmine and did not stain with PAS. The same staining features were evident in Case 10, except that the layer was PAS-positive.

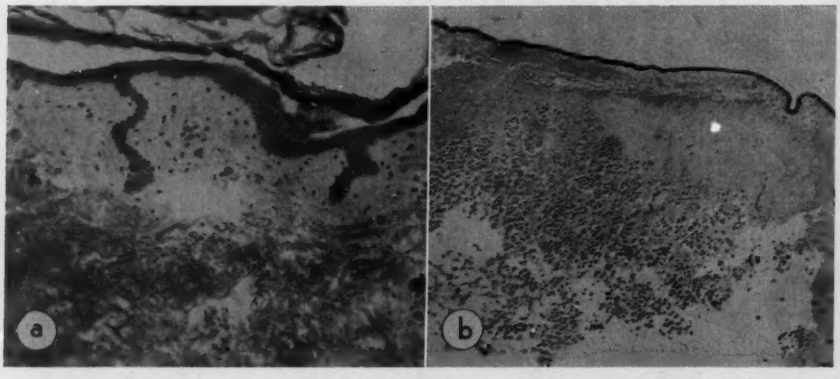

FIG. 2. (Case 1) **a.** Section of skin, showing atrophy of epithelium and abundant ground substance in the upper corium (mucoid substance) (hematoxylin and eosin; ×120). **b.** Section of vocal cord; abundant mucoid substance in the subepithelial stroma and between the muscle fibers (hematoxylin and eosin; ×6).

The vocal cord was examined histologically only in Case 1. The subepithelial stroma appeared basophilic, markedly edematous, and contained finely fibrillar and granular material. Mucous glands and muscle fibers were compressed by this material (Fig. 2b). Blood vessel walls were also edematous.

The submucosa of the trachea was edematous in 2 cases studied (Cases 6 and 8).

Representative sections of the parotid gland were obtained in 2 instances (Cases 9 and 10). Many small groups of mucous cells were present which contained material staining basophilic with hematoxylin, pink with mucicarmine, and PAS-positive. The inter-

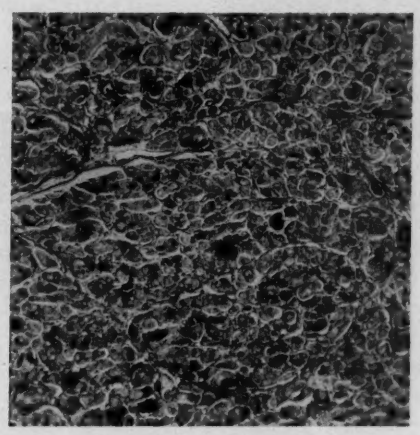

FIG. 3. (Case 6) Myocardium in cross section, showing numerous dark PAS-positive areas within the muscle fibers. Basophilic degeneration of the myocardium (PAS; ×80).

calated ducts in Case 10 contained a homogeneous eosinophilic substance which was PAS and mucicarmine positive. The mucous cells noted in the parotid may possibly represent mucinous degeneration of serous cells.

Heart and aorta. Many of the myocardial fibers appeared swollen and contained a striking accumulation of pale blue-staining material in the form of large, irregular vacuoles (Fig. 3). These vacuolated areas were brilliantly PAS-positive. Mallory's PTAH stain showed the myofibrils of these involved fibers to be widely separated and distorted by the intracellular accumulations. This striking feature, basophilic degeneration of the myocardium, was present to extreme degree. In addition, there were small amounts of interstitial pale blue-staining material in many areas. In Case 4 occasional groups of tissue mast cells were scattered throughout the interstitial tissue, chiefly perivascular in location, but sometimes present between the muscle fibers. The mast-cell granules stained metachromatically a dark purple color and were PAS-positive.

In the few sections of the aorta available for study there were intimal changes characteristic of atherosclerosis.

Intestinal tract. A constant feature was the histologic alteration of the intestine, particularly the colon. Numerous accumulations of tissue mast cells were scattered throughout the muscularis propria and the subserous connective tissue (Fig. 4a). The mast cells were well granulated; the granules stained metachromatically a dark purple with toluidine blue and were PAS-positive. They were especially abundant in perivascular areas.

Areas of interstitial mucoid substance were often present where the mast cells were most abundant, appearing basophilic with hematoxylin and faintly PAS-positive. Located within this ground substance were finely fibrillar, loosened collagen fibers. Fibers of the muscularis propria were frequently swollen and the fibers were separated by the mucoid material (Fig. 4b).

Skeletal muscle. Sections of muscle were obtained from the anterior abdominal wall in

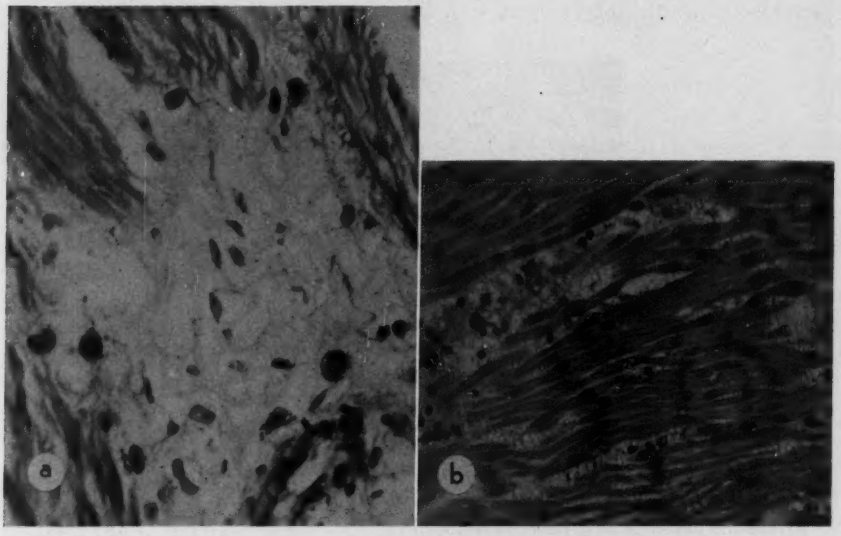

FIG. 4. **a.** (Case 4). Tissue mast cells in interstitial tissue of muscularis propria of colon (toluidine blue; ×400). **b.** (Case 2) Section of colon, showing swelling of fibers of muscularis propria as well as intercellular edema (hematoxylin and eosin; ×360).

3 cases. In cross section, a few fibers exhibited semilunar PAS-positive areas in the periphery.

Bone marrow, liver and spleen. Anemia had been present to some degree in each patient. Examination of the sternal bone marrow during life had revealed hypocellularity in Cases 9 and 10. Significant hemosiderosis of marrow, liver and spleen was present in 5 cases.

Pituitary myxedema

Histologic and histochemical features of the 9 cases of primary myxedema were lacking in the 3 non-endocrine control cases, as well as in the single case of pituitary myxedema (Case 7), all studied similarly.

The thyroid gland in the latter instance contained only a slightly increased amount of fibrous tissue. Many small follicles of various size were present, often minute, lined by flat epithelium and containing eosinophilic colloid. (A good response by this gland to administration of thyroid-stimulating hormone had been demonstrated in a test during life.) The pituitary was markedly atrophic, especially the anterior lobe. The adrenal cortex was very atrophic; its layers were difficult to define and the cells were markedly

depleted of lipid material. There was pronounced glandular atrophy of the testis; tubules were atrophic and degenerated, interstitial cells very sparse, and spermatogenesis almost absent. Sections of skin, heart and intestine exhibited no characteristic changes. There was a single mucosal ulceration of the duodenum, however. Hemosiderosis was evident in liver and spleen. The sternal bone marrow during life had been hypoplastic.

DISCUSSION

The changes described in the thyroid gland represent a terminal stage of thyroid atrophy. Some of the features suggest a previous chronic thyroiditis; however, they may possibly represent inflammatory changes secondary to a degenerative process. Many of the changes in the other endocrine glands could have been due to the ages of the subjects or the terminal state prior to death. Thrombosis of the adrenal veins, in 1 case, may have been unrelated to myxedema. The testis, in 1 case, microscopically had the structural features reported by Marine (12).

The cutaneous alterations were located primarily in the dermis, and represented the deposition of mucoid substance in that area (18). Similar alterations were noted in the tongue and vocal cords. The findings in the parotid gland were unusual, because this organ normally has a wholly serous secretion. Perhaps a mucinous parotid secretion occurs in myxedema. Large quantities of mucin in the parotid gland have been previously reported in 1 case and in monkeys following thyroidectomy (3).

The degree of sclerosis of the coronary and of the major arteries seemed unusually great, despite the advanced ages of these subjects. The severe degree of basophilic degeneration of the myocardium seemed characteristic, although this feature cannot be considered specific for the myxedematous state (19–24). Serous effusions were common in these cases; pericardial effusions were, however, not present.

Marked distention of the colon has previously been observed in myxedema and is a common manifestation (25). The accumulations of mast cells in the subserous connective tissue were a prominent feature, as were the adjacent depositions of mucoid substance. Arvy (26) has reported many mast cells in the mesentery of the rat treated with thiourea.

Metachromasia with toluidine blue has been said to be induced by any substance of large molecular weight with free acid radicals. Metachromatic material largely occurs extracellularly, and this fact coupled with other evidence has led to the belief that it represents acid mucopolysaccharides (27). PAS positivity is indicative primarily of the presence of insoluble 1,2-glycolic groups, typical of polymer carbohydrates (28).

Asboe-Hansen (29) stated that mucinous substances, predominantly mucopolysaccharides combined with protein, and tissue mast cells accumulate in the dermis of patients with myxedema. The mucopolysaccharides are chiefly hyaluronic acid and chondroitin-sulfuric acid. A characteristic of myxedema was said to be the accumulation of tissue mast cells in the

connective tissue generally. However, data on human pathologic material other than skin were not reported by him. He believes that mast cells secrete heparin and perhaps a precursor of hyaluronic acid. An increase in the thyroid-stimulating hormone content of serum and urine has been shown in myxedema and the changes in this disease are attributed by Asboe-Hansen to this factor. However, Altshuler (30) believes that the factor stimulating the connective tissue in myxedema may favor deposition of mucopolysaccharides and also may stimulate differentiation of mast cells. In accord with either hypothesis are the findings described in our cases of primary myxedema and their absence in the single case of pituitary myxedema. In the latter, thyroid-stimulating hormone activity was known to be absent.

SUMMARY

Nine clinically recognized cases of adult primary myxedema, 1 case of pituitary myxedema, and 3 cases of nonendocrine disease (controls) have been studied by means of routine autopsies and histochemical methods.

In the 9 cases of primary myxedema, the thyroid gland was atrophied and presented alterations, perhaps inflammatory in nature, either primary, or secondary to a degenerative process.

Mucoid substance, identified histochemically, was present in the upper dermis and in the subepithelial stroma of the tongue and vocal cords. The parotid gland apparently had produced a mucinous secretion.

There was a significant degree of coronary arteriosclerosis and cardiac hypertrophy in each case. Many individual myocardial fibers contained vacuolated areas which were basophilic when stained with hematoxylin and were also PAS-positive. Basophilic degeneration of the myocardium is characteristic but not specific in myxedema.

There was marked distention of the colon in 4 cases. In all cases, numerous tissue mast cells were scattered throughout the muscularis propria and subserous connective tissue of the intestine. Areas of interstitial mucoid substance were often present where the mast cells were most abundant. Fibers of the muscularis frequently appeared swollen.

The foregoing alterations were not observed in the tissues of the single case of pituitary myxedema (panhypopituitarism).

In myxedema, the widespread changes in connective tissue manifested by deposition of acid mucopolysaccharide and proliferation of mast cells are possibly related to activity of the thyroid-stimulating hormone.

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STUDIES IN NONMYXEDEMATOUS HYPOMETABOLISM. II. TURNOVER OF I^{131}-LABELED THYROXINE AFTER INTRAVENOUS INFUSION*†

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THE term "euthyroid hypometabolism," has been utilized to describe a group of patients having basal metabolic rates below −20 per cent in association with a variety of clinical symptoms suggestive of hypothyroidism but in whom other customary parameters of thyroid function are within normal limits. The concept that the low metabolic rate might be the result of a defect in the peripheral metabolism of thyroxine received support from our experience in the treatment of such patients with triiodothyronine or with mixtures of triiodothyronine and thyroxine (1, 2). In each of 4 cases, the basal metabolic rate was persistently elevated following the oral administration of triiodothyronine, alone or with thyroxine, although equivalent doses of desiccated thyroid had been ineffective.

Previous studies in such patients failed to reveal any abnormality of thyroidal function as measured by the serum levels of protein-bound iodine and cholesterol, or by the 24-hour thyroidal uptake of I^{131}. With the availability of I^{131}-labeled *l*-thyroxine, it became possible to investigate more directly the peripheral metabolism of thyroxine. The utility of this technique has been demonstrated (3, 4) in a small number of subjects with normal thyroid function, hyperthyroidism, myxedema, panhypopituitarism and hypermetabolism without thyroid disease, but no such data are available in patients with euthyroid hypometabolism. The present communication reports the results of preliminary experiments designed to study the peripheral metabolism of thyroid hormone in patients with this syndrome.

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MATERIALS AND METHODS

Twelve euthyroid hypometabolic patients were studied. Nine were males aged 16 to 61; the 3 females were 22 to 41 years of age. In each case, careful clinical and laboratory examination had established the euthyroid state. Five of the 12 were asymptomatic. Seven had a variety of manifestations suggestive of hypothyroidism. Four complained of marked fatigue, pain in the joints, and ease of weight gain. Two were apparently normal male members of a subfertile marriage. Seven presented the facial and periorbital puffiness which is frequently seen in euthyroid hypometabolic patients. The basal metabolic rates in the whole group ranged from −21 to −44 per cent (average, −27 per cent) (5). The serum protein-bound iodine level ranged from 4.3 to 8.0 micrograms (μg.) per 100 cc. (average, 5.8 μg.) (6). The serum cholesterol level varied from 160 to 330 mg. per 100 cc. (average, 225 mg.) (7, 8). In 4 of the 12 patients, the serum cholesterol concentration was above 250 mg. per 100 cc. One of these 4 was a 60-year-old man with xanthelasma and angina pectoris: in none was there other evidence of hypothyroidism. The *in vitro* two-hour red blood cell uptake of I^{131}-labeled triiodothyronine from the patient's own plasma averaged 14.2 per cent per 100 hematocrit (range 10.2–21.0) (9). The 24-hour thyroidal I^{131} uptake in 6 of the 12 patients ranged from 28 to 35 per cent (average 32 per cent). All of these values are consistent with the euthyroid state.

It should be noted that, although these patients satisfied the requirements for inclusion in this study, *i.e.* low basal metabolic rate with normal thyroid function, their response to the administration of desiccated thyroid, thyroxine or triiodothyronine had not been determined prior to the infusion study. In view of the known prolonged effect of administered thyroid hormone on pituitary-thyroid function, it was felt necessary to carry out the I^{131}-thyroxine infusions in patients whose status was uncomplicated by previous thyroid therapy. It was recognized that by these broad terms of selection the study would include a heterogeneous group of subjects with low basal metabolic rates, and that some of them would be normal persons whose metabolic rates were at the low end of the normal distribution. However, known endocrine and other causes of low basal metabolism were excluded.

The data on the 12 patients were compared with those on 10 euthyroid volunteers with normal basal metabolism. Nine of the 10 were female. Their ages ranged from 21 to 36 years. The basal metabolic rates varied from +3 to −18 per cent (average, −7 per cent). The serum protein-bound iodine level ranged from 4.0 to 6.9 μg. per 100 cc. (average, 5.5 μg.) The red blood cell uptake of triiodothyronine varied from 11.2 to 16.4 per cent per 100 hematocrit (average, 13.5 per cent). All of the patients were asymptomatic and apparently healthy.

I^{131}-labeled *l*-thyroxine was obtained from a commercial source.[1] An aliquot of each shipment was analyzed by paper chromatography in multiple solvent systems before administration to the patients. Estimates of purity of the labeled thyroxine indicated that the infused material contained less than 10 per cent of non-thyroxine radioactivity. Samples that were less pure were discarded. A dose of 1 to 3 μg. of *l*-thyroxine labeled with 40 to 75 μc. of I^{131} was administered intravenously from a tuberculin syringe. Suitable standards were prepared simultaneously in quadruplicate from dilutions of I^{131}-thyroxine from the same shipment, to which 2–3 drops of concentrated serum albumin were added. Correction was made for radioactivity residual in the syringe used for the infusion and for preparation of the standards. At 24-hour intervals thereafter during periods up to eleven days (average, nine days) thyroidal radioactivity was measured with a

[1] Abbott Laboratories, Chicago, Illinois.

single, collimated scintillation counter (sodium iodide-thallium activated crystal, 1-inch diameter) at a distance of 30 cm. Correction for body scatter was made by measuring a suitable radioactive mock-up in air and against the patient's neck. Radiation in the thigh was also measured at a distance of 30 cm. and was subtracted from total neck radioactivity in an attempt to correct for non-thyroid sources. Duplicate or triplicate aliquots of plasma, and of 24-hour collections of urine and (in some cases) feces, were counted in a well-type scintillation counter. The uptake in the thyroid gland and the radioactivity in plasma and excreta were expressed as percentages of the dose administered. Data were plotted on semilogarithm paper (Fig. 1). The disappearance of plasma radioactivity was exponential after initial equilibration (twenty-four to forty-eight hours) and was expressed as the slope of the line which visually best fitted the data (Fig. 1). From this, the half-time of disappearance of plasma thyroxine ($t_{\frac{1}{2}}$) or the length of time (days) required for plasma radioactivity to diminish by 50 per cent was obtained. The percentage of thyroxine turned over per day

$$(k) = \frac{\ln 2 \times 100}{t_{\frac{1}{2}}} = \frac{0.693}{t_{\frac{1}{2}}} \times 100.$$

The volume of distribution of thyroxine ("thyroxine space" or TDS) was calculated by two methods. In one, the total injected radioactivity was divided by the plasma concentration extrapolated to the time of infusion from the slow portion of the disappearance curve. In the second method, the residual thyroxine (injected dose minus the percentage accumulated in the thyroid and that excreted in the urine and feces) was divided by the plasma concentration (3). The volume (V) turned over per day was equal to TDS×k. The extrathyroidal thyroxine (ETT) was the product of TDS×PBI γ/100 cc.×10. The quantity of thyroxine degraded daily (D) was obtained from the product of V×PBI γ%/100 cc.×10.

RESULTS

The results are shown in Tables 1 and 2. The half-time ($t_{\frac{1}{2}}$) of disappearance of plasma radioactivity in the hypometabolic patients (Table 1) ranged from 6.3 to 10.1 days and averaged 8.3 days (S.D. 1.1; S.E. 0.32). In the normal metabolic group (Table 2) it ranged from 6.6 to 7.8 days and averaged 7.18 days (S.D. 0.43; S.E. 0.13). The difference between these means is significant at the level, $P < .01$ but $> .005$. The mean percentage turned over per day (k) was 8.5 and 9.7 per cent, respectively. The thyroxine distribution space in the hypometabolic group varied from 7 to 17 liters and averaged 10.6 liters (S.D. 4.5), whereas that in the control group varied from 7 to 12 liters and averaged 9.94 liters (S.D. 1.9). The wide range in the former group is notable; 5 patients had a distribution space between 12.8 and 17.0 liters. The volumes (V) turned over per day averaged, respectively, 996 and 949 cc. and the extrathyroidal thyroxine averaged 625 and 512 μg. The quantity of thyroxine degraded per day in the hypometabolic group ranged from 20 to 87.5 μg. and averaged 49.7 μg. (S.D. 23.8); in the control group the range was 36 to 61 μg., with an average of 50.9 μg. (S.D. 8.6).

DISCUSSION

The thyroxine turnover observed in our normal control subjects was in the range previously reported for persons with normal thyroid function (3, 4). In contrast, a statistically significant decrease in the disappearance rate of infused thyroxine was observed in the hypometabolic group.

All patients in our control and hypometabolic group were euthyroid. The serum protein-bound iodine level was normal in each case. Results of I^{131} tracer studies, when performed, were normal. The *in vitro* red blood cell uptakes of labeled triiodothyronine (a measure of the relative binding affinities for thyroid hormone of the plasma protein and tissue components) were not significantly different in the two groups and were within the euthyroid range as determined in our laboratory. The sole metabolic criterion for classifying a patient in one or other of the two groups was the basal metabolic rate, which in the one group averaged −27 per cent and in the other −7 per cent. However, it must be emphasized that the control group, with the exception of 1 man aged 24, consisted of young asymptomatic female volunteers. On the other hand, the hypometabolic group was more heterogeneous regarding age, sex and symptomatology. Data currently available do not suggest that the differences observed between the two groups can be related to age or sex.

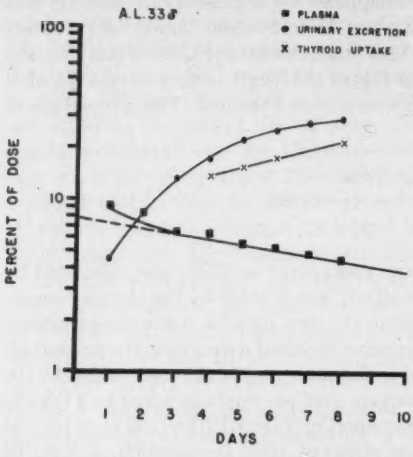

FIG. 1. Plasma I^{131}-thyroxine level (per cent dose per liter), cumulative urinary I^{131} excretion (per cent dose) and thyroidal I^{131} uptake (per cent dose) in Patient A. L., Case 2.

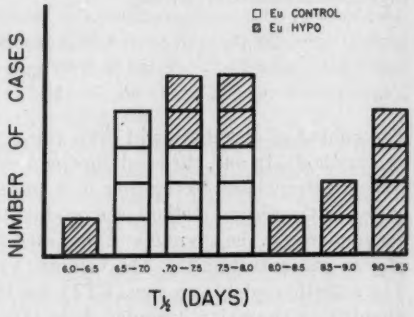

FIG. 2. Distribution of the half-time of disappearance of I^{131}-thyroxine in 12 euthyroid hypometabolic patients (cross-hatched squares) and 10 control patients (open squares).

TABLE 1. THYROID FUNCTION STUDIES IN 12 EUTHYROID HYPOMETABOLIC PATIENTS

Patient		BMR	Serum PBI	RBC*	Serum chol.	Thyroidal I^{131} uptake	$t_{\frac{1}{2}}$**	k	TDS	V	ETT	D	U	T
No. & name	Age & sex	(%)	(μg./100 cc.)		(mg./100 cc.)	(%)	(days)	(%)	(liters)	(cc.)	(μg.)	(μg.)	(max.)	(max.)
1 L.M.	41 F	−22	7.7	10.2	330	35.0	9.6	7.2	5.0	360	—	27.7	77.0	—
2 A.L.	41 M	−32	6.2	12.4	169	35.0	9.4	7.4	10.5	740	650	46.0	—	—
3 B.K.	22 F	−44	4.3	10.8	212	—	9.8	7.1	6.1	467	264	20.0	65.0	25.0
4 I.F.	33 M	−25	7.5	14.5	176	—	8.5	8.2	17.0	1,350	700	61.0	55.0	37.0
5 J.W.	28 M	−25	7.1	12.0	—	29.0	9.4	7.4	16.0	1,210	1,160	86.0	45.0	34.0
6 M.S.	60 M	−38	5.8	21.0	295	33.0	6.3	11.0	7.0	750	406	43.0	73.0	28.0
7 K.K.	16 M	−22	5.0	16.6	174	33.2	7.8	8.9	5.0	445	—	22.0	—	—
8 L.L.	34 M	−21	5.2	15.4	231	—	7.0	9.9	9.3	920	480	47.0	67.0	33.0
9 E.D.	26 F	−25	6.0	13.7	202	28.0	8.0	8.7	8.0	696	360	37.5	83.0	31.0
10 G.M.	45 M	−25	5.0	—	218	—	8.5	8.2	12.8	1,045	640	52.3	54.5	24.5
11 M.S.	26 M	−27	5.6	17.3	267	—	7.1	9.8	16.0	1,560	896	87.5	49.5	31.0
12 B.H.	29 M	−25	4.6	—	196	—	7.9	8.8	15.0	1,320	690	61.0	61.0	30.0
Average	33.4	−27	5.8	14.4	223	32.0	8.3	8.5	10.6	905	625	49.3		

* I^{131}-triiodothyronine uptake in % per 100 hematocrit (9).
** $t_{\frac{1}{2}}$ = half-time of disappearance of plasma I^{131}-thyroxine.
k = percentage of thyroxine turned over per day.
TDS = volume of distribution of thyroxine.
V = volume turned over per day.
ETT = extrathyroidal thyroxine.
D = quantity of thyroxine degraded daily.
U = Urinary excretion (%).
T = Thyroidal accumulation (%).

TABLE 2. THYROID FUNCTION STUDIES IN 10 NORMAL CONTROL SUBJECTS

Patient		BMR	Serum PBI	RBC*	$t_{\frac{1}{2}}$**	k	TDS	V	ETT	D	U	T
No. & Name	Age & Sex	(%)	(μg. /100 cc.)		(days)	(%)	(liters)	(cc.)	(μg.)	(μg.)	(max.)	(max.)
1. L.W.	22 F	− 8	6.9	11.2	7.3	9.5	7.0	670	405	39.0	—	—
2. M.M.	22 F	−18	5.2	13.0	7.2	9.6	7.2	692	372	36.0	77.0	22.0
3. A.H.	24 M	− 6	5.0	15.6	6.9	10.0	11.0	1,100	550	55.0	47.0	45.0
4. M.M.	29 F	− 3	4.6	11.6	7.8	9.1	12.0	—	—	—	—	—
5. E.T.	36 F	− 7	6.0	—	7.6	8.6	9.2	838	462	50.2	65.0	37.5
6. N.P.	27 F	+ 3	6.8	12.7	7.8	8.9	10.0	890	680	60.5	59.0	29.5
7. B.G.	24 F	± 0	5.4	—	7.0	9.9	9.8	960	530	52.0	40.0	61.0
8. H.G.	24 F	−13	5.8	—	6.6	10.5	10.0	1,050	580	61.0	52.0	29.0
9. G.T.	21 F	−12	5.0	16.4	6.8	10.1	10.7	1,090	535	54.5	57.2	—
10. S.E.	31 F	−10	4.0	14.1	6.8	10.0	12.5	1,250	500	50.0	54.0	42.0
Average	26	− 7	5.5	13.5	7.18	9.6	9.9	949	512	50.9		

* For key to symbols, see Table 1.

Although the mean thyroxine turnover rate was slower in the hypometabolic group, there was overlapping between the individual values in each group (Fig. 2). The most rapid turnover ($t_{\frac{1}{2}} = 6.3$ days) was found in a 60-year-old man with angina pectoris and xanthelasma whose basal metabolic rate was repeatedly −38 per cent and serum cholesterol level 295 mg. per 100 cc.; however, his serum protein-bound iodine level was 5.8 μg. per 100 cc., and 24-hour thyroidal I^{131} uptake 33 per cent. It should be noted that his red blood cell uptake of I^{131}-triiodothyronine was 21 per cent (in the hyperthyroid range). No difference between the means of the two groups was noted in the other parameters of thyroxine metabolism measured, *i.e.*, the thyroxine distribution space, the daily volume of thyroxine turned over, the extrathyroidal thyroxine, and particularly the thyroxine degradation rate. However, 3 patients of the hypometabolic group had depressed rates of thyroxine degradation of 20.0 to 27.7 micrograms daily—approximately half the normal value.

Decreased rates of thyroxine turnover and degradation have also been noted in cases of primary myxedema and panhypopituitarism (3, 4), but both of these conditions were excluded in the patients described here. Studies of thyroxine turnover in other hypometabolic states are in progress. Data are available in 3 euthyroid males with testicular failure, in 2 of whom the basal metabolic rate was −20 per cent. These patients will be described in detail elsewhere (10). In 2 of these 3 hypogonadal patients, a decreased rate of thyroxine turnover was observed. However, the male patients in our euthyroid hypometabolic group had no evidence of testicular failure.

In planning this investigation, it was hoped to obtain an objective means of distinguishing persons with a low metabolism representing merely the

low end of the normal distribution of metabolic rates from those with abnormalities in the metabolic pathway of thyroxine. The present series of 12 hypometabolic patients, which included 3 whose turnover rate was distinctly normal, is not sufficiently large to allow us to correlate any known variables with thyroxine turnover rate. It is our impression that the hypometabolic patients whose half-times of disappearance of plasma radioactivity were 7.5 days or less had no symptoms referable to hypometabolism. No correlation was found between turnover rate and the facial puffiness which is frequently present in this condition. Four of the 12 hypometabolic patients were treated, following these infusion studies, with triiodothyronine or a combination of triiodothyronine and thyroxine. All had relatively prolonged half-times ($t\frac{1}{2}$ = 7.8 to 9.8 days); all responded to therapy by elevation of the basal metabolic rate. We have not yet, however, had the opportunity to observe the response to triiodothyronine in a euthyroid hypometabolic patient with a more normal thyroxine turnover time. Finally, no evidence is yet available to indicate whether the slowed turnover rate seen in some hypometabolic patients is specific for thyroxine, or will be shown to exist also for other iodinated derivatives such as triiodothyronine, or labeled proteins such as I^{131}-albumin. Such studies are now in progress.

The present limited number of observations point to a possible abnormality in thyroxine metabolism in euthyroid hypometabolic patients. They illustrate the utility of infusions of labeled thyroxine in disclosing altered metabolism of thyroid hormone, when customary parameters of thyroid function are within normal limits. Many additional observations in similar patients will be required, however, before the relationship between thyroxine metabolism and lowered metabolic rates is clarified.

SUMMARY

1. I^{131}-labeled *l*-thyroxine was infused into 12 euthyroid hypometabolic patients and 10 normal control subjects. From the curve of thyroxine disappearance, the thyroid and urinary radioactivity, and the level of plasma protein-bound iodine, calculations were made of the half-time of plasma radioactivity, the thyroxine distribution space, volume of distribution, extrathyroidal thyroxine, and thyroxine degradation rate.

2. The mean half-time of disappearance of thyroxine from plasma was slower (8.3 days) in the euthyroid hypometabolic group than in the control group (7.18 days) ($P < .01$ but $> .005$). No significant difference was noted in mean thyroxine space, volume of turnover, and thyroxine degradation rate. Three patients had low thyroxine degradation rates of 20–27 micrograms per day.

3. The present limited number of observations point to a possible ab-

normality in thyroxine metabolism in euthyroid hypometabolic patients. Additional observations in similar patients may clarify in detail the relationship between thyroxine metabolism and lowered metabolic rates.

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Editorial Reviews

CLINICAL SIGNIFICANCE OF SERUM BUTANOL-EXTRACTABLE IODINE*†

THE level of serum butanol-extractable iodine (BEI) or thyroxine-like iodine is a specific diagnostic criterion for evaluating thyroid function and therapy (1). Measurement of the protein-bound or serum-precipitable iodine (PBI, SPI)[1] concentration provides a useful and convenient tool for assaying thyroid function in most patients (2–4), especially children (5) and those with cardiovascular disease in whom the basal metabolic rate is difficult to interpret. With increased experience in the use of the PBI test, it was found that in some instances the values obtained were at variance with the clinical status of the patient (1). Since it is generally believed that the active circulating hormone of the thyroid gland is "thyroxine-like" (6), and since thyroxine is extractable from the plasma in butanol and remains dissolved therein after alkaline washing of the butanol, a method for measuring the "butanol-extractable iodine" was introduced in 1951 (1). Experience with this test has shown it to have appreciable advantages over the older PBI or SPI methods. After therapeutic administration of inorganic (7) or radioactive iodine (8) the resultant high level of PBI may be erroneously interpreted. BEI values correlate better with the clinical status of thyroid function.

NORMAL VALUES

The normal range of serum BEI is more sharply defined than that of the SPI. The maximum BEI level for adults is at least 1 gamma (microgram) per 100 ml. less than the maximum usually cited for the PBI (9). The minimum BEI level for children is higher than that for adults—a significant observation in the diagnosis of juvenile hypothyroidism (5, 10). This higher

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† The experimental data were obtained in a study aided by a grant from the United States Public Health Service.

[1] SPI is used in this article to indicate measurements in this laboratory of iodine precipitated with zinc hydroxide (3, 4). PBI refers to determinations made elsewhere in which the agents used to precipitate proteins vary from laboratory to laboratory.

For SPI's and BEI's determined in this laboratory, digestion, distillation and measurement of the iodide ion were the same for both procedures. Therefore differences between SPI and BEI emphasize the discrepancy resulting from extracting the BEI rather than precipitating the organic iodine compounds of blood serum with proteins. The differences are not influenced by the variations which occur from one laboratory to another.

minimum BEI level in childhood is supported by the observation that in euthyroid children there is a higher thyroidal uptake of radioactive iodine than in euthyroid adults (11).

A compilation of serum BEI levels in groups of subjects from birth through maturity is presented in Figure 1. Excluded were persons whose

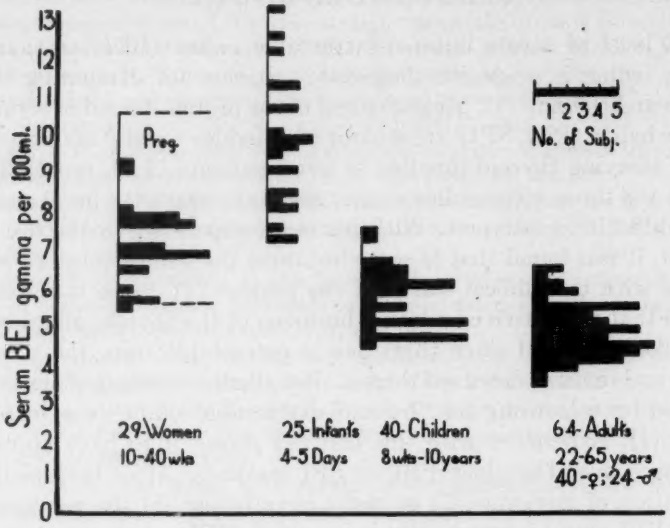

FIG. 1. Histogram of serum BEI levels in groups of euthyroid persons ranging from infants to adults.

At the left, the broken lines denote the range derived from the results of 114 BEI determinations in 33 euthyroid pregnant women between the tenth and fortieth weeks of pregnancy; and each unit in the black bars (see key in chart) represents the average value for each of 29 women.

In the other age groups each unit in the black bars represents the BEI level in a single subject.

BEI level might have been altered by hypoproteinemia, malnutrition, acute infection (10), liver disease or antecedent diagnostic or therapeutic use of organic iodine compounds (1, 9). During the first hours after birth the serum BEI concentration is similar to that in the mother's blood (12). In 19 of 20 comparisons, the BEI content of maternal serum at delivery and of cord blood serum agreed within 1.6 gamma per 100 ml. in our series (13), and almost as closely in the series of Friis and Secher (14). At the age of 4 or 5 days, the infants' BEI levels were higher than those in cord serum, and lay between 7.2 and 13.2 gamma per 100 ml. (13). The euthyroid range for children from 6 weeks to 10 years of age was 4.5 to 7.3 gamma per 100 ml. (10). This exceeded the normal range for 64 nonpregnant adults (9)—3.2 to 6.4 gamma per 100 ml. (mean 4.7; S.D. $\pm$0.72).

When SPI concentrations were measured routinely in our laboratory, values for euthyroid and hyperthyroid subjects overlapped at the maximum of the normal range (4). This overlap was reduced when the BEI concentration was determined. To verify the maximum normal serum BEI concentration, careful clinical evaluations were made in 171 adults, 21 to 92 years in age, whose BEI levels were between 5.0 and 8.0 gamma per 100 ml. (9). In all of the 23 persons who, by clinical criteria, were judged to be hyperthyroid the BEI values were above 6.5 gamma per 100 ml. Of the 148 adults judged to be euthyroid only 3 (each of whom had cardiovascular symptoms) had BEI levels as high as 6.8 gamma per 100 ml. These data confirm the validity of 6.4 gamma per 100 ml. as a reasonable value for the maximum concentration of BEI in the euthyroid adult range. In normal women after the tenth week of pregnancy the BEI levels were between 5.5 and 10.5 gamma per 100 ml. (Fig. 1) (13, 15).

IODINE COMPOUNDS MEASURED

To claim that serum BEI determinations are more precise than PBI determinations implies that the exact chemical nature of the organic iodine compounds included in measurement of the BEI is more accurately delineated than is the nature of the iodine compounds precipitated with protein. "Thyroxine-like" (1, 6, 9) is the best description of the organic iodine compounds which are included in BEI measurements, in spite of the results of recent investigations of analogues of thyroxine and investigations of thyroxine-binding to proteins (16). Recoveries of iodine from thyroxine in aqueous alkaline solution alone, or when added to serum *in vitro*, were between 88 and 103 per cent—adding the amounts found in 1 ml of serum (1). Triiodothyronine, triiodothyroacetic acid and tetraiodothyroacetic acid added *in vitro* to serum yielded recoveries of iodine which were as complete as those from thyroxine. However, when triiodothyronine in maintenance doses was administered *in vivo*, no increase in the concentration of serum BEI could be detected. Moreover, when as much as 32 gamma per 100 ml. of diiodotyrosine iodine or 1,000 gamma per 100 ml. of inorganic iodine was added *in vitro* to serum (1), none was recovered. Ingbar *et al.* (17) in their study of butanol-extractable I^{131} found similar percentile recoveries of thyroxine I^{131} and virtual exclusion of the I^{131} linked with potassium iodide or diiodotyrosine. In our original SPI studies (3), 84 per cent of the iodine of diiodotyrosine was recovered. Thus there is a quantitatively important difference between the specificity of the BEI as compared with the SPI determination. The BEI technique does not eliminate contamination from organic iodine compounds[2] given for diagnostic or therapeutic purposes (9).

[2] See Appendix for essential technique in obtaining sera for BEI determinations.

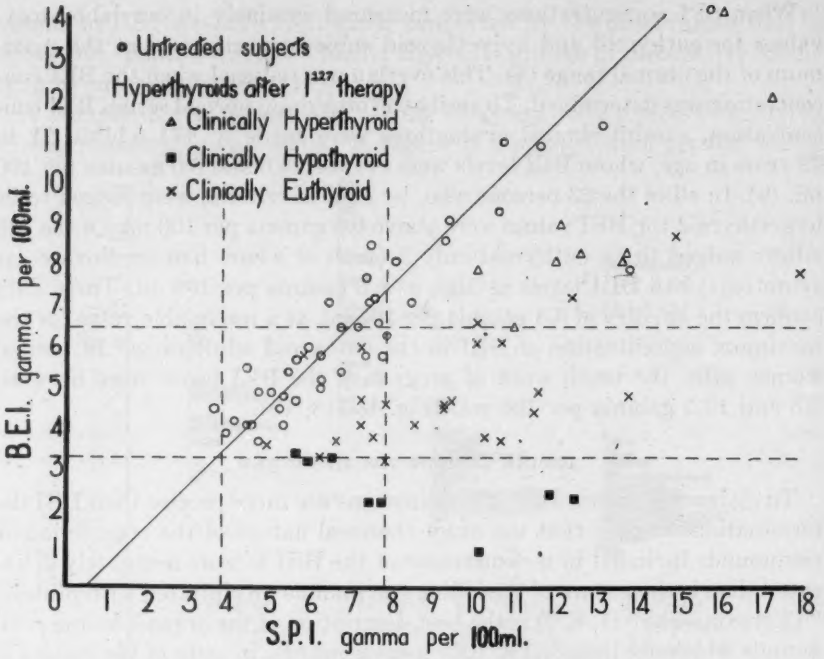
• Untreated subjects
Hyperthyroids after I^{127} therapy
△ Clinically Hyperthyroid
▪ Clinically Hypothyroid
× Clinically Euthyroid
B.E.I. gamma per 100ml.
S.P.I. gamma per 100ml.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 2. Comparison of serum BEI and SPI determinations.

Circles represent 41 comparisons in 107 untreated subjects, some of whom were hyperthyroid. (See text for detailed explanation of figure.) Overcrowding of points in the normal range prevented inclusion of more than 41 of the 107 comparisons.

Other symbols (see key in chart) represent values for hyperthyroid patients receiving Lugol's solution alone or with propylthiouracil.

EFFECT OF IODINE THERAPY

SPI and BEI concentrations were measured in 107 sera from normal and from clinically hyperthyroid subjects before and during medical treatment (1), as shown in Figure 2. Broken vertical lines represent the normal range for SPI (3.8 to 7.8 gamma per 100 ml. (4)) and broken horizontal lines represent the normal range for BEI (3.2 to 6.4 gamma per 100 ml. (9)). The diagonal line drawn at an angle of 45 degrees starts from a point representing a BEI concentration of 0 and an SPI concentration of 0.5 gamma per 100 ml., as the average excess of SPI over BEI was 0.5 gamma per 100 ml. in the sera of 84 nonpregnant adults, 14 pregnant women, and in 9 sera from cord blood or infant's blood. None of the subjects had a history of previous ingestion of potassium iodide, Lugol's solution or iodine-

containing medication for asthma. The 41 open circles on Figure 2 represent normal persons and patients with untreated hyperthyroidism and nontoxic goiter (1). There is good agreement between the SPI and BEI values, with 1 exception; in this instance, a BEI value of 7.8 gamma corresponded to an SPI value of 13.7 gamma per 100 ml. in a patient with an enormous goiter which began during childhood. This person is mentioned later in connection with other untreated patients in whom there were significant differences between SPI and BEI values.

In Figure 2, the triangles, crosses and squares represent the comparisons of SPI and BEI values in 3 classes of patients who were under treatment for hyperthyroidism with Lugol's solution alone or with propylthiouracil. In the group with clinical hypothyroidism several SPI values were in the hyperthyroid or euthyroid range but the BEI values were within, or near the hypothyroid range. This difference persisted for months in 2 patients under special observation. In many of the patients who were clinically euthyroid during treatment the SPI values were in the hyperthyroid range but those for BEI were in the euthyroid range. Two such results (elevated BEI level of about 8 gamma per 100 ml., with SPI level almost twice as high) were observed in a patient whose hyperthyroid symptoms seemed controlled by therapy. This 56-year-old patient, whose huge goiter had increased in size during twenty-eight years, had been born in Hungary where endemic goiter was prevalent. Although the BEI levels in this exceptional patient were higher than anticipated clinically, they were much closer to the normal level than were the SPI values.

Thyroid dysfunction in the pregnant woman may influence profoundly the health and mental development of her offspring (13). The administration of inorganic iodine during pregnancy may elevate spuriously the serum concentration of PBI in both mother and infant, so that the degree of activity of the thyroid gland cannot be evaluated from this determination. Three pregnant women taking Lugol's solution had PBI levels which were 4.3, 4.5 and 5.6 gamma per 100 ml. higher than the BEI levels, which were in accord with the clinical impression of thyroid activity. SPI levels of 21 and 38 gamma per 100 ml. were significantly elevated as compared with the BEI levels in 2 goitrous newborn infants who had not been given inorganic iodine, but whose mothers had received massive doses of potassium iodide during pregnancy. The occurrence of high serum concentrations of PBI in goitrous infants born to mothers who had taken inorganic iodine during pregnancy has been described by Bongiovanni and co-authors (18).

From Figure 2 it is evident that, when a patient has received inorganic iodine, the concentration of SPI may be falsely elevated by 2 to 10 gamma per 100 ml. as compared with the concentration of BEI. In such instances,

the BEI value agrees more closely with the clinical status than does the PBI value. As a result, the SPI value may not truly reflect the clinical state of thyroid activity. This spurious elevation of the SPI level seems to be a metabolic result of the administration of inorganic iodine to the patient, and is not a technical error. Danowski found that even 14 washings would not reduce the SPI concentration to the level in sera obtained before the ingestion of iodide (7). *In vitro* addition of potassium iodide to serum did not increase the concentration of SPI. Elevation of the SPI level caused by administration of iodide was reported by Danowski to persist for months (7). Such an elevation may also be caused by iodide ingested (perhaps unknown to the patient) in any of the many preparations which include it. There are over 300 pharmaceutical products which might cause iodide contamination, and many of them appear in superficially unimpressive form as vitamins or "tonics." Hydovitz and Rose (19) described a clinically hypothyroid man with a serum PBI level within the normal range up to two hundred and seventy-five days after ceasing to take an organic iodine preparation for asthma, although the BEI level (1 gamma per 100 ml.) was in the hypothyroid range and was in accord with the clinical picture. These authors suggested that in their patient there was an enzymatic error in the synthesis of metabolically active thyroxine-like substances, either inborn or acquired during twelve years of therapy with large doses of an iodine-containing medication for asthma.

In studies involving recognition of naturally occurring metabolic aberrations causing differences between SPI and BEI values, persons who have received therapeutic doses of radioactive iodine should be excluded. Many articles present evidence that after therapeutic doses of radioactive iodine the plasma contains non-thyroxine-like organic iodine compounds such as diiodotyrosine or mono-iodotyrosine and possibly thyroglobulin. Rall (8) has summarized conditions in which the various radioactive iodine tests are most accurate and the conditions in which the results may be erroneous. When these tests are not valid because of previous therapy with radioactive iodine or after continuous intake of inorganic iodine, measurement of the serum BEI is a more reliable test.

EFFECT OF THYROID DISORDERS

When the exogenous factors of iodide ingestion and I^{131}-irradiation of the thyroid are excluded, certain states of thyroid dysfunction have been found to induce significant elevations of the serum level of PBI as compared to that of BEI. The endogenous causes may be associated with familial, metabolic or thyroid dysfunctions. The number of patients in the present study is too limited for any correlation of these enzymatic disturbances with size or duration of goiter or other metabolic disorders. How-

ever, the incidence of unusual iodinated compounds in the blood stream or of abnormal radioactive iodine thyroidal uptakes in hypothyroid patients with familial goiters has been reported by Hutchison and McGirr (20), Stanbury *et al.* (21) and others (22–24). In 4 patients of our series, 3 of whom had goiters, the SPI levels were higher by 3 to 6 gamma per 100 ml. than the BEI levels. In each instance the PBI value was not in agreement with the clinical diagnosis but the BEI value confirmed the clinical impression. In an 84-year-old woman with a huge goiter over the sternum the BEI level was 7.8 gamma per 100 ml.—a concentration compatible with the clinical impression of masked hyperthyroidism and advanced heart failure; the SPI level (13.7 gamma per 100 ml.) was higher than suggested by the mildness of the thyrotoxic symptoms. Two children had goiters, physical and mental retardation, and thyroidal radioactive iodine uptakes not in agreement with the clinical symptoms of hypothyroidism. One of these, a 6-year-old girl, had an initial SPI level of 4.9 gamma per 100 ml. (euthyroid range) but a BEI level of 1.9 gamma per 100 ml. (hypothyroid range); she has received triiodothyronine with resulting improvement in growth rate and reduction in the size of her goiter. In the other, a 14-year-old mentally and physically retarded boy, enlargement of the left lobe of the thyroid had been noted for eleven years; chromatographic studies following radioactive iodine in tracer doses revealed thyroxine and monoiodotyrosine in the plasma; the level of SPI exceeded that of the BEI by 6 gamma per 100 ml.

Gribetz, Talbot and Crawford (25) reported that in adolescent girls with Hashimoto's syndrome the BEI values were in better agreement with the clinical impressions of hypo- or euthyroidism than were the results of radioactive iodine tracer tests, or the PBI values (which were sometimes elevated to the hyperthyroid range). When the patients who were clinically hypothyroid received replacement therapy, the excess of PBI over BEI decreased simultaneously with reduction in the size of the goiter; it seemed as if this syndrome were the result of lack of functioning thyroid hormone in the blood stream and consequent increased production of thyrotropin. In studying another type of thyroiditis (giant-cell, or De Quervain), Ingbar and Frienkel (26) found that in 2 women the levels of SPI exceeded those of the BEI. In these patients BEI determinations were made only on the initial blood samples. At this time there were no clinical signs of thyrotoxicosis although the BEI values were above the normal range. The reason for the discrepancy between the elevated BEI values and the normal clinical appearance in these patients is not clear.

The metabolic conditions in which the level of PBI in an untreated patient exceeds the level of BEI are not observed frequently in this area, even in goitrous or hypothyroid persons. In 5 patients treated locally, 2 of whom

were clinically hypothyroid and 3 of whom were elderly persons with goiters, the SPI values were in satisfactory agreement with the BEI values. There were no reports of exceptional familial metabolic disorders in these 5 patients.

SUMMARY

Determination of the serum level of butanol-extractable iodine as a test of thyroid function has two advantages: 1) it is not altered by the administration of iodides, and 2) it measures the thyroxine-like compounds of the serum. Discrepancies between PBI and BEI values may sometimes be useful in diagnosing cases of thyroiditis or nontoxic goiter in which the gland is unable to secrete normal thyroid hormone. In patients who have been treated with radioactive iodine, the thyroid may secrete iodinated compounds not found normally in the blood stream. The clinical progress of these patients cannot be evaluated with determinations of the thyroidal radioactive iodine uptake or the serum PBI level, but determination of the BEI level, not being affected by the metabolic abnormality of the thyroid gland, can serve as an accurate criterion of thyroid function during treatment.

Acknowledgments

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Appendix

Many sera contain unexpectedly high concentrations of iodine as a result of the increased use of organic iodine-containing compounds for roentgenography and the frequent incorporation of iodine in pharmaceutical preparations. Such iodine may not only ruin the determination of BEI in the particular case involved, but may contaminate the syringe used for drawing the blood, and the analytical equipment. A protective isolation procedure to reduce contamination had to be instituted in our laboratory. All needles and syringes used in taking blood for this analysis are kept in the BEI laboratory and issued to the wards as needed. The needle and syringe are returned to the laboratory with the specimen and not released for sterilization and re-use until the BEI measurement indicates the absence of contamination. The blood is collected in lustroid centrifuge tubes which are discarded after the first use. All stirring rods, tubes and pipettes for iodine analyses are kept separately from the apparatus used in the same room for other blood analyses. The screening test of Malkin (27) has proved useful in detecting gross contamination, but moderate elevations of iodine concentration cannot be detected, since the method is sensitive only to concentrations over 100 gamma per 100 ml.

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CAN RADIATION CAUSE THYROID CANCER?*†

IN 1950 it was first suggested that there might be some relationship between irradiation of the thymus gland and cancer of the thyroid (1). In a survey of the cases of thyroid cancer seen at the Memorial Hospital in New York from 1932 to 1948, 28 patients were found to have histologic evidence of thyroid cancer before the age of 18 years. Ten of these 28 had been irradiated over the chest in infancy for symptoms thought to be due to enlarged thymus. The authors stated at that time that "to propose a cause-and-effect relationship between thymic irradiation and the development of cancer would be quite unjustified on the basis of data at hand when one considers the large number of children who have had irradiation to an 'enlarged thymus.' However, the potential carcinogenic effects of irradiation are becoming increasingly apparent, and such relationships as those of thymic irradiation in early life and the subsequent development of thyroid or thymic tumors might be profitably explored."

This is a review of clinical and experimental work, developed since the initial report, which confirms and extends the observation that there is a relationship between irradiation in infancy and childhood and the subsequent development of thyroid cancer.

CLINICAL DATA

Following the original observation on the possible association of thymic irradiation and thyroid cancer, an attempt was made to obtain a history of radiation in young patients with thyroid cancer. In 2 subsequent papers (2, 3), 42 cases of cancer of thyroid in children or young adults were listed. A history of previous irradiation over the chest was obtained in only 1 of this group.

In contrast to these negative findings Clark (4) reported that in all of the 15 children who had come under his care for treatment of thyroid cancer, previous therapeutic amounts of roentgen-rays had been administered to the head and neck (9 patients) or upper thorax (6 patients) during the first six years of life. Although a control group was not available for comparison, the probability of finding by chance 15 patients who had received x-ray therapy in a total series of 15 cases of thyroid cancer is less than 1 in 100, even if one assumes that 50 per cent of the general population received therapeutic doses of x-rays in childhood. Clark suggested that the increased incidence of thyroid cancer in the younger age group might be related to the increased use of x-rays for treatment of benign childhood diseases.

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The first controlled study on the possible association of x-ray therapy over the chest in infancy, and the later development of cancer was that of Simpson and associates (5). They reviewed the histories of 1,400 children who had received x-radiation to the thymus in infancy. As controls, 1,795 siblings who had not received x-ray therapy were studied. In 6 children of the irradiated group cancer of the thyroid developed, whereas in none of the siblings in the non-irradiated group was cancer of the thyroid observed during the same period.

Simpson and Hempelmann (6) reviewed their patients who had received x-ray therapy to the thymus and added case material with the following current findings:

	No. of patients	Thyroid cancer	Thyroid adenomas
Treated by x-ray	2,333	11	9
Siblings (no x-ray treatment)	2,622	0	1

Av. age at time of x-ray therapy—1 month.
Av. age at time of cancer—12.2 years.
Av. length of follow-up for group—14 years.

Using the New York State incidence data for 1949–51, the expected number of cases of all types of cancer in the treated group would be 3.6; actually, 21 cases were observed, including 7 cases of leukemia. The expected number of cases among the siblings would be 3.8; actually, 6 cases were observed. The difference in the sibling group is not significant, but the probability of finding by chance the increase noted in the treated group is less than 1 in 1,000.

The high incidence of leukemia in the x-ray treated group is further evidence that the carcinogenesis was probably the result of exposure to x-rays. Two recent British studies are of interest in regard to radiation leukemogenesis and the increased risk of radiation carcinogenesis in the young patient. Court-Brown and Doll (7) postulated that there may be no threshold for the development of leukemia after exposure to x-rays. Stewart and associates (8) found an increased incidence of cancer, including leukemia, in children whose mothers had x-ray examinations of the abdomen during pregnancy.

According to the report of Simpson and co-workers, none of the children in whom thyroid cancer developed after thymic irradiation "received a dose of less than approximately 180 r to the thyroid." Although their series included only children who had received x-ray therapy for an "enlarged thymus," mention was made of additional patients who had received x-ray therapy for other conditions and who later had thyroid cancer. Wilson and

Asper (9) reviewed 37 patients with thyroid cancer under the age of 25 and found that 17 had been given therapeutic doses of x-rays in childhood, but in only 4 for thymic enlargement. In a control group of 31 patients with thyroid adenoma in childhood, only 1 had received x-ray treatment for any cause.

In contrast to these reports of the relationship between therapeutic doses of x-rays in infancy and the later development of thyroid cancer, there is a paucity of published data on x-ray carcinoma of the thyroid in the adult. Quimby and Werner (10) conducted a survey for possible cases of thyroid cancer following x-ray therapy for benign thyroid disease. On the basis of radiologists' records, the authors concluded that the "possibility of late malignant degeneration is negligible following roentgen therapy for toxic goiter." However, they did list 4 patients with carcinoma of other structures in the neck (larynx, trachea and esophagus) twenty years after x-ray therapy for thyrotoxicosis.

Goolden (11) collected 42 cases of radiation carcinoma of the pharynx and found that, although the x-ray therapy in most instances had been given for the treatment of thyrotoxicosis, the increased incidence of cancer of the pharynx was not accompanied by an increased incidence of cancer of the thyroid gland. The mean latent period for the development of pharyngeal cancer after x-ray therapy was twenty-five years.

We recently studied 328 patients who received x-ray therapy for thyrotoxicosis in the Washington, D.C. area more than twenty-five years ago. These patients had been treated by one group of radiologists, and the x-ray dosage was sufficient to have resulted in some changes in the skin in the majority of patients. Although no evidence of thyroid cancer was found, the limited number of subjects precludes statistical analysis.

There is the further difficulty of interpretation, in that, when compared to the younger age group, these adult patients presumably had abnormal thyroid function at the time of x-ray therapy. Thyrotoxic patients may have increased resistance to the development of radiation cancer of the thyroid.

There have been no reports of thyroid cancer developing in patients treated with I^{131}. Since it has been found that carcinoma of the thyroid occurs in about 0.4 per cent of patients with exophthalmic goiter, the incidence of thyroid cancer will have to exceed this figure in the I^{131}-treated group if I^{131} is to be suspected of being a carcinogenic agent. If the incidence remains well below this figure one might, as has been suggested (12), make a theoretic case for the use of I^{131} in preventing the development of thyroid cancer in hyperthyroidism.

Clinical reports concerning the relationship between x-ray therapy and the development of thyroid cancer might be summarized as follows: 1) there is strong circumstantial evidence that therapeutic x-radiation in infants

may be an etiologic factor in the thyroid cancer of childhood and adolescence, and 2) there is no present evidence that either x-ray irradiation or I^{131}-irradiation can cause thyroid cancer in adult thyroid tissue.

ANIMAL EXPERIMENTAL DATA

Hall (13), reviewing the work of Bielschowsky, and Purves and Greisbach, postulated the dual action of initiation and promotion in thyroid carcinogenesis. He suggested an *initiating* phase in which a carcinogen produces latent tumor cells, and a *promoting* phase in which endogenous TSH stimulation (goitrogen-produced) causes these cells to develop into visible tumors.

Doniach (14) extended this concept in a series of experiments which confirmed the dual role of radiation and goitrogens in the development of experimental thyroid cancer in the rat. Doniach first established the dose (30 μc. of I^{131}) which resulted in the development of adenomas in 10 out of 16 rats. With larger, more destructive doses of I^{131}, the incidence of tumors declined sharply. When the 30-μc. dose of I^{131} was followed by methylthiouracil continued for twelve to fifteen months, the adenomas increased in number, and 5 thyroid cancers developed in 20 rats treated.

When 1,100 rads of x-ray therapy was administered to the rat thyroid, followed by prolonged methylthiouracil therapy, thyroid cancer developed in 7 out of 21 rats. In only 1 of 13 animals given 1,100 rads of x-rays but no methylthiouracil did thyroid cancer develop.

As a measure of radiation sensitivity, determinations were made of the inhibition of goitrogenesis in weanling rats given various doses of I^{131} at the age of 30 days and tested with a goitrogenic challenge given four months later. There was no difference in sensitivity compared to that previously observed in the adult rat. Thymectomy of weanling rats did not result in any detectable change in thyroidal I^{131} turnover.

In summary, the experimental work of Doniach indicates an increased incidence of thyroid cancer in the rat treated with 30 μc. of I^{131} or 1,100 rads of x-rays, particularly if the radiation be followed by prolonged stimulation of the pituitary (by administration of goitrogen).

DISCUSSION

Doniach (14d) has suggested that a small dose of x-radiation may act as the initiating factor and that the subsequent "normal" growth and development of the thyroid from infancy to puberty may be the promoting factor in the production of thyroid cancer in childhood. The dose of external x-rays or other radiation would not then be as important as the time at which it is administered, since the mature adult thyroid tissue is apparently reasonably resistant to the development of radiation cancer.

Oliner and associates (15) have presented data on thyroidal I^{131} uptake

and serum PBI^{131} levels to indicate that the thyroid gland in children up to the age of 4 years is normally in a state of "hyperactivity" when compared to that of the euthyroid adult. Sclare (16) has studied the histologic pattern of the thyroid gland in the newborn infant and found it suggestive of "heightened physiological activity." It would be of interest to follow these changes throughout the normal thyroidal growth period extending to puberty, at which time the thyroid gland reaches its adult weight and structure.

In addition to restricting unnecessary external irradiation, conservative judgment would also interdict the use of tracer I^{131} in infancy and early childhood. A dose of I^{131} resulting in the thyroidal uptake of 20 μc. distributed equally throughout a 10-gram thyroid gland would deliver 200 rads to the thyroid. Due to the spotty distribution of I^{131}, some areas of the thyroid receive much higher doses of radiation from the same amount of administered I^{131}. These differences in distribution of radioactivity might account for the relatively low dose of I^{131}-irradiation, compared to x-irradiation, which is carcinogenic in the rat.

With regard to the therapeutic use of I^{131} in the adult patient (over 20 years of age), there is no reasonable argument for restriction, on the basis of present data. We administer desiccated thyroid after I^{131} treatment of the young adult patient in order to inhibit excessive production of thyrotropin by the pituitary, since increased production of TSH may be one of the factors in the development and growth of thyroid tumors. It is possible that a significant increase in the frequency of thyroid cancer in the group of I^{131}-treated patients will still be found. "This would not necessarily be an argument against using I^{131}, any more than the known risk of death after operation is an argument against surgery. The problem is rather to weigh the risks from alternative forms of treatment in each patient" (17).

SUMMARY

There have been repeated reports indicating an association between therapeutic x-irradiation of benign disease in childhood and the later development of thyroid cancer in a small, but highly significant, number of the children so treated.

The experimental work indicates that the administered irradiation is potentiated by endogenous endocrine activity, in the development of thyroid cancer. Such a mechanism may account for the increased risk after the use of x-rays in the infant or growing child, compared to that in the adult.

This review of clinical and radiobiologic data on the relationship between radiation and thyroid cancer suggests: 1) restriction of the use of I^{131}, including tracer doses, in the young patient until results of further studies of radiation carcinogenesis in the neonatal and childhood period

are available, and 2) no restriction of the use of therapeutic doses of I^{131} when indicated in the adult patient, since there is no evidence, clinical or experimental, that radiation can cause cancer in adult thyroid tissue.

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20, and 21, 1958.*

The Committee on Local Arrangements is Dr. Roberto Escamilla, Chairman, Dr. Donald E. Bernstein, Dr. Peter H. Forsham, Dr. Minnie B. Goldberg, Dr. Gilbert S. Gordan, Dr. Francis S. Greenspan, Dr. C. H. Li as members of the committee.

All Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily in the Colonial Ballroom, and in addition there will be simultaneous afternoon sessions in the Italian Room. The annual dinner is scheduled for Friday, June 20 at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All reservations for rooms must be made through the AMA Housing Bureau, c/o Convention and Tourist Bureau, Civic Auditorium, San Francisco, California, after January 1, 1958. No reservations will be accepted before that date. If possible, official application forms as published in the Journal of the American Medical Association should be used. Arrival and departure dates must be stated.

Final program, membership card and advance registration forms will be sent on May 15, 1958 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Rulon W. Rawson, 444 E. 68th St., New York 21, N. Y., not later than *February 1, 1958. It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that those will be presented at the meeting*—in order that they may be of reference value and suitable for printing in the program. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. Reference, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

2. The title heading must be arranged as follows:

   Line 1. Title, not to exceed fifteen words;

Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter perfect, since there will be no opportunity for proofreading by the authors.

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—

Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1958 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the St. Francis Hotel, San Francisco, California, on *June 17, 18 and 19, 1958.*

THE VAN METER PRIZE AWARD FOR 1958

The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the Association, which will be held in the St. Francis Hotel, San Francisco, California, June 17, 18 and 19, 1958, provided essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, N. Y., *not later than February 1, 1958.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the winning essay by the author if it is possible for him to attend.

RECIPIENTS OF AWARDS FOR 1957

RECIPIENT OF VAN METER PRIZE

In 1930, Dr. S. D. Van Meter offered a prize of $300.00 to be awarded to the essayist presenting the best work on the subject of "Goiter—Especially its Basic Cause." As a founding member, Dr. Van Meter enthusiastically supported the object of the Association, which is given in the constitution "—the acquisition and spread of knowledge of the thyroid gland and its diseases." Upon his death in 1934, the Association continued the Award and named it after its originator. The Van Meter Prize Award has been made each year with the exception of 1943, 1944 and 1945.

Throughout the years, essays have been received from many foreign countries as well as our own. Competition has been keen, and the committee chosen to review the manuscripts has frequently found it difficult to name a winner. In addition to the winner, it has been customary for the committee to select two papers for Honorable Mention. This year, Honorable Mention Awards have been given to Farahe Maloof, M.D. for his paper entitled "The Uptake and Metabolism of S^{35} Labeled Thiourea and Thiouracil by the Thyroid and Other Tissues" and to S. B. Barker, Ph.D. for his paper entitled "In Vitro Action of Thyroxine Analogs on Succinate and Malate Oxidation."

The Winner of the Award this year was *Deborah Doniach, M.D.* who, in collaboration with *Dr. I. M. Roitt*, presented a paper entitled "Auto-immunity in Hashimoto's Disease and Its Implications."

Dr. Doniach is a native of Switzerland but received her medical training in the Royal Free Hospital Medical School, University of London. In 1947 she became a Lecturer in Clinical Biochemistry at the Courtauld Institute, Middlesex Hospital. She has received degrees in Chemical Pathology and has held hospital appointments at the Royal Free Hospital and Middlesex Hospital in London.

Dr. Roitt, who collaborated with Dr. Doniach, was born in London and received his education at Oxford, Balliol College, in Chemistry. In 1952 he received his Doctorate in Chemistry and a year later the degree of Bachelor of Arts in Physiology. Since 1953, he has been associated with the Courtauld Institute of Biochemistry, Middlesex Hospital. His particular interest has been the mode of action of growth inhibitors and problems of protein biosynthesis and auto-immunity.

RECIPIENT OF AWARD FOR LONG AND MERITORIOUS SERVICE

At its annual meeting in Columbus, Ohio in the spring of 1951, the American Goiter Association established a Long and Meritorious Service

Award for those of its members who had contributed significantly to its scientific and business affairs. The first Award was made to Dr. André Crotti, who is an internationally famous member of the Association, distinguished for his many contributions to our knowledge of the thyroid gland. In subsequent years, the Award was given to Drs. James Hill, Frank Lahey, Nelson Percy, John Pemberton, Allen Graham, J. H. Means, V. E. Chesky, Sam Haines, and (posthumously). George Shivers.

The committee appointed to select a candidate for the Award this year has unanimously chosen *Merrill N. Foote, M.D.* of Brooklyn, New York. Dr. Foote is a native of Tompkins County, New York, and received his education at Cornell University and the College of Physicians and Surgeons of Columbia University. He served during the First World War as a volunteer with Mrs. Harry Payne Whitney's unit of the American Red Cross, and after our entry into the war he became a Captain in the Medical Corps of our Army.

Dr. Foote is Director of Surgery at St. John's Episcopal Hospital in Brooklyn and Chief Surgeon at Carson C. Peck Memorial Hospital. He is Attending or Consulting Surgeon to more than fifteen additional hospitals in the Brooklyn and Long Island area.

He has long been affiliated with the American College of Surgeons, of which he is a Governor; he has been an officer since 1938, and President since 1950, of the Brooklyn and Long Island Chapter. He is a Past-President of the American Goiter Association.

In addition to his affiliations with many other medical and surgical societies. Dr. Foote has been a distinguished leader in the Bellport Yacht Club. His activities in many sportsmen, hunting and gun clubs attest to his versatile interests and love of life.

FOURTH INTERNATIONAL CONFERENCE ON GOITER

The London Thyroid Club and the American Goiter Association announce that the Fourth International Conference on Goiter will be held in London, England in 1960. Scientific sessions will be held in the Royal College of Surgeons on *July 6, 7 and 8, 1960.*

Those who desire to submit abstracts of papers to be considered for the program should write to either *Selwyn Taylor, F.R.C.S.*, 3 Roedean Crescent, Roehampton, London S.W. 15, England, or *John C. McClintock, M.D.*, 149½ Washington Avenue, Albany 10, N. Y., U.S.A.

Travel and hotel accommodations may be secured from Thomas Cook and Sons, Ltd.

A very few Honoraria will be available for travel assistance. Further particulars may be obtained from either of the above.